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# CONTENTS INHALT MATIÈRES

(CYTOLOGIA Vol. 10)

## No. 1-2 (Nov. 1939)

	Paginae
<b>Meurman, O. and Therman, E.</b> , Studies on the Chromosome Morphology and Structural Hybridity in the Genus <i>Clematis</i> . . . . .	1-14
<b>Seshachar, B. R.</b> , Inversion of a Centric Segment in a Chromosome of <i>Ichthyophis glutinosus</i> (Linn) . . . . .	15-17
<b>Northen, H. T. and MacVicar, R.</b> , Studies of Protoplasmic Structure in <i>Spirogyra</i> . VI. Effects of sound and electricity on elasticity . . . .	18-22
<b>Raghavan, T. S. and Venkatasubban, K. R.</b> , Studies in the Cappariadaceae: V. The cytology of <i>Crataeva religiosa</i> Forst. . . . .	23-31
<b>Matthey R.</b> , La loi de Robertson et la formule chromosomiale chez deux Lacertiens: <i>Lacerta ocellata</i> Daud., <i>Psammodromus hispanicus</i> Fitz. . . . .	32-39
<b>Braun, W.</b> , Disturbances in the Process of Cell-Division in the Pupal Wing of the Flour-Moth <i>Ephestia kühniella</i> as Result of Heat-Treatment. . .	40-43
<b>Küster, E.</b> , Über Vakuolenkontraktion und Anthozyanophoren bei <i>Pulmonaria</i> . . . . .	44-50
<b>Tanaka, N.</b> , Chromosome Studies in Cyperaceae, IV. Chromosome number of <i>Carex</i> species . . . . .	51-58
<b>Mensinkai, S. W.</b> , Cytological Studies in the Genus <i>Gladiolus</i> . . . . .	59-72
<b>Sugiura, T.</b> , Chromosome Numbers in Plumbaginaceae . . . . .	73-76
<b>Howard, H. W.</b> , The Cytology of Autotetraploid Kale, <i>Brassica oleracea</i> . . . . .	77-87
<b>Nishiyama, I.</b> , Cytogenetical Studies in <i>Avena</i> . II. On the progenies of pentaploid <i>Avena</i> hybrids . . . . .	88-100
<b>Nishiyama, I.</b> , Cytogenetical Studies in <i>Avena</i> . III. Experimentally produced eu- and hyperhexaploid aberrants in oats . . . . .	101-104
<b>Northen, H. T.</b> , Studies on the Protoplasmic Nature of Stimulation and Anesthesia . . . . .	105-112
<b>Suzuki, K.</b> , Regeneration of Gonads in <i>Plecoglossus altivelis</i> after Spawning Season . . . . .	113-126
<b>Satô, D.</b> , Cyto-genetical Studies on <i>Tricyrtis</i> , II. Karyotype analysis in <i>Tricyrtis</i> and <i>Brachycyrtis</i> with special reference to SAT- and nucleolar chromosomes . . . . .	127-157
<b>Wada, B.</b> , Experimentelle Untersuchungen lebender Zellen in der Teilung. IV. Die Einwirkung des Dampfgemisches von Ammonia und Chloroform auf die Mitose bei den <i>Tradescantia</i> -Haarzellen . . . . .	158-179
<b>Yasui, K.</b> , Karyological Studies on <i>Iris japonica</i> Thunb. and Its Allies. . . . .	180-188
<b>Raghavan, T. S. and Venkatasubban, K. R.</b> , Studies in the Indian Scilleae. II. The cytology of <i>Scilla indica</i> Baker. . . . .	189-204
<b>Sugiura, T.</b> , Studies on the Chromosome Numbers in Higher Plants III. . . . .	205-212
<b>Kuwada, Y.</b> , Chromosome Structure. A Critical Review . . . . .	213-256
<b>Maheshwari, P.</b> , Recent Advances in Microtechnique II. The paraffin method in plant cytology . . . . .	257-281

## No. 3 (Jan. 1940)

<b>Makino, S.</b> , On the Chromocentre Observed Through the Mitotic Cycle of Somatic Cells in <i>Drosophila virilis</i> . . . . .	283-293
<b>Fujii, S.</b> , An Abnormal Staining Capacity of the Sixth Salivary Gland Chromosome of a Strain of <i>Drosophila virilis</i> . . . . .	294-301

	Paginae
<b>Takamine, N.</b> , On the Plasmolysis Form in <i>Allium cepa</i> with Special Reference to the Influence of Potassium Ion upon It . . . . .	302-323
<b>Sugiura, T.</b> , Studies on the Chromosome Numbers in Higher Plants. IV.	324-333
<b>Ito, T. und Kubo, M.</b> , Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Über die Ganglienzellen des menschlichen Darmes, mit besonderer Berücksichtigung auf die Nissl-substanz . . . . .	334-347
<b>Tanaka, N.</b> , Chromosome Studies in Cyperaceae, VI. Pollen development and additional evidence for the compound chromosome in <i>Scirpus lacustris</i> L. . . . .	348-362
<b>Sugiura, T.</b> , Studies on the Chromosome Numbers in Higher Plants. V.	363-370
<b>Yamaha, G. und Suita, N.</b> , Über die Wirkung verschiedener Pufferlösungen auf die <i>Spirogyra</i> -Zellen . . . . .	371-381
<b>Matsuura, H. und Haga, T.</b> , Chromosome Studies on <i>Trillium kamschatcicum</i> Pall. VIII. On the mitosis-meiosis relationship . . . . .	382-389
<b>Matsuura, H.</b> , Chromosome Studies on <i>Trillium kamschatcicum</i> Pall. XII. The mechanism of crossing-over . . . . .	390-405
<b>Nishina, Y., Sinotô, Y. and Satô, D.</b> , Effects of Fast Neutrons upon Plants, II. Abnormal behavior of mitosis in <i>Vicia faba</i> . . . . .	406-421

#### No. 4 (Jun. 1940)

<b>Klein, B. M.</b> , Verschiedenartige Entformungen entquellender Ciliaten . .	423-433
<b>Lebedeff, G. A.</b> , Failure of Cytokinesis During Microsporogenesis in <i>Zea mays</i> Following Heat Treatment . . . . .	434-442
<b>Jensen, H. W.</b> , Further Studies on Sex Linked Chromosome Abnormalities.	443-449
<b>Osima, T.</b> , Cyto-genetic Studies of <i>Drosophila montium</i> (Report I) . . .	450-457
<b>Nishina, Y., Sinotô, Y. and Satô, D.</b> , Effects of Fast Neutrons Upon Plants, III. Cytological observations on the abnormal forms of <i>Fagopyrum</i> and <i>Cannabis</i> . . . . .	458-466
<b>Srinath, K. V.</b> , Morphological and Cytological Studies in the Genus <i>Calceolaria</i> Part II. Meiosis in diploid and aneuploid <i>Calceolarias</i> . . . .	467-491
<b>Kuwada, Y. and Nakamura, T.</b> , Behaviour of Chromonemata in Mitosis IX. On the configurations assumed by the spiralized chromonemata.	492-515
<b>Singh, B. N., Sampath, S. and Bansal, R. K.</b> , Two Problems in the Interpretation of Meiosis in Plants . . . . .	516-523
<b>Oka, T. B.</b> , Striation Pattern of the Epithelium-Cells of Yolk-Sac Membrane and Embryonic Body of <i>Oryzias latipes</i> . . . . .	524-528
<b>Sinotô, Y. and Satô, D.</b> , Basikaryotype and Its Analysis . . . . .	529-538
<b>Kusunoki, S.</b> , Neue Beobachtungen über Protoplasmaströmung bei <i>Hydrilla verticillata</i> . . . . .	539-544
<b>Oka, T. B.</b> , Extrusion of a Nucleolus <i>in toto</i> , Found in the Ovarian Oocytes of <i>Holothuria monacaria</i> . . . . .	545-550
<b>Yasui, Y.</b> , Cytogenetic Studies in Artificially Raised Interspecific Hybrids of <i>Papaver</i> . IX. On the bivalents-association in the meiosis of the PMC of <i>Papaver somniferum</i> . . . . .	551-557
<b>Sugiura, T.</b> , Chromosomes Studies on Papaveraceae with Special Reference to the Phylogeny . . . . .	558-576
<b>Takamine, N.</b> , Studies on the Influence of Ultraviolet Rays on the Cytoplasm, Tested by the Plasmolysis-Form Method . . . . .	577-587

List of New Publications Received.

Liste der zugegangenen Neuerscheinungen.

Liste des publications nouvelles reçues. . . . . (1)-(12)

# INDEX

## (CYTOLOGIA Vol. 10)

	Paginae
<b>Braun, W.</b> , Disturbances in the Process of Cell-Division in the Pupal Wing of the Flour-Moth <i>Ephestia kühniella</i> as Result of Heat-Treatment. . .	40 - 43
<b>Fujii, S.</b> , An Abnormal Staining Capacity of the Sixth Salivary Gland Chromosome of a Strain of <i>Drosophila virilis</i> . . . . .	294-301
<b>Howard, H. W.</b> , The Cytology of Autotetraploid Kale, <i>Brassica oleracea</i> . . .	77 - 87
<b>Ito, T. und Kubo, M.</b> , Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Über die Ganglienzellen des menschlichen Darmes, mit besonderer Berücksichtigung auf die Nissl-substanz . . . . .	334-347
<b>Jensen, H. W.</b> , Further Studies on Sex Linked Chromosome Abnormalities. . .	443-449
<b>Klein, B. M.</b> , Verschiedenartige Entformungen ent quellender Ciliaten . .	423-433
<b>Küster, E.</b> , Über Vakuolenkontraktion und Anthozyanophoren bei <i>Pulmonaria</i> . . . . .	44 - 50
<b>Kusunoki, S.</b> , Neue Beobachtungen über Protoplaststromung bei <i>Hydrilla verticillata</i> . . . . .	539-544
<b>Kuwada, Y.</b> , Chromosome Structure. A Critical Review . . . . .	213-256
<b>Kuwada, Y. and Nakamura, T.</b> , Behaviour of Chromonemata in Mitosis. IX. On the configurations assumed by the spiralized chromonemata. . .	492-515
<b>Lebedeff, G. A.</b> Failure of Cytokinesis During Microsporogenesis in <i>Zea mays</i> Following Heat Treatment . . . . .	434-442
<b>Maheshwari, P.</b> , Recent Advances in Microtechnique II. The paraffin method in plant cytology . . . . .	257-281
<b>Makino, S.</b> , On the Chromocentre Observed Through the Mitotic Cycle of Somatic Cells in <i>Drosophila virilis</i> . . . . .	283-293
<b>Matsuura, H.</b> , Chromosome Studies on <i>Trillium kamschaticum</i> Pall. XII. The mechanism of crossing-over . . . . .	390-405
<b>Matsuura, H. and Haga, T.</b> , Chromosome Studies on <i>Trillium kamschaticum</i> Pall. VIII. On the mitosis-meiosis relationship. . . . .	382-389
<b>Matthey, R.</b> , La loi de Robertson et la formule chromosomiale chez deux Lacertiens: <i>Lacerta ocellata</i> Daud., <i>Psammodromus hispanicus</i> Fitz. . .	32 - 39
<b>Mensinkai, S. W.</b> , Cytological Studies in the Genus <i>Gladiolus</i> . . . . .	59 - 72
<b>Meurman, O. and Therman, E.</b> , Studies on the Chromosome Morphology and Structural Hybridity in the Genus <i>Clematis</i> . . . . .	1 - 14
<b>Nishina, Y., Sinotô, Y. and Satô, D.</b> , Effects of Fast Neutrons upon Plants, II. Abnormal behavior of mitosis in <i>Vicia faba</i> , . . . . .	406-421
<b>Nishina, Y., Sinotô, Y. and Satô, D.</b> , Effects of Fast Neutrons upon Plants, III. Cytological observations on the abnormal forms of <i>Fagopyrum</i> and <i>Cannabis</i> . . . . .	458-466
<b>Nishiyama, I.</b> , Cytogenetical Studies in <i>Avena</i> . II. On the progenies of pentaploid <i>Avena</i> hybrids. . . . .	88-100
<b>Nishiyama, I.</b> , Cytogenetical Studies in <i>Avena</i> . III. Experimentally produced eu- and hyperhexaploid aberrants in oats. . . . .	101-104
<b>Northen, H. T.</b> , Studies on the Protoplasmic Nature of Stimulation and Anesthesia . . . . .	105-112

	Paginae
Northern, H. T. and MacVicar, R., Studies of Protoplasmic Structure in <i>Spirogyra</i> . VI. Effects of sound and electricity on elasticity . . . . .	18-22
Oka, T. B., Striation Pattern of the Epithelium-Cells of Yolk-Sac Membrane and Embryonic Body of <i>Oryzias latipes</i> . . . . .	524-528
Oka, T. B., Extrusion of a Nucleolus <i>in toto</i> , Found in the Ovarian Oocytes of <i>Holothuria monacaria</i> . . . . .	545-550
Osima, T., Cyto-genetic Studies of <i>Drosophila montium</i> (Part I) . . . . .	450-457
Raghavan, T. S. and Venkatasubban, K. R., Studies in the Capparidaceae: V. The cytology of <i>Crataeva religiosa</i> Forst. . . . .	23-31
Raghavan, T. S. and Venkatasubban, K. R., Studies in the Indian Scilleae. II. The cytology of <i>Scilla indica</i> Baker. . . . .	189-204
Satô, D., Cyto-genetical Studies on <i>Tricyrtis</i> . II. Karyotype analysis in <i>Tricyrtis</i> and <i>Brachycyrtis</i> with special reference to SAT- and nucleolar chromosomes . . . . .	127-157
Seshachar, B. R., Inversion of a Centric Segment in a Chromosome of <i>Ichthyophis glutinosus</i> (Linn) . . . . .	15-17
Singh, B. N., Sampath, S. and Bansal, R. K., Two Problems in the Interpretation of Meiosis in Plants . . . . .	516-523
Sinotô, Y. and Satô, D., Basikaryotype and its Analysis . . . . .	529-538
Srinath, K. V., Morphological and Cytological Studies in the Genus <i>Calceolaria</i> . Part II. Meiosis in diploid and aneuploid <i>Calceolarias</i> . . . . .	467-491
Sugiura, T., Chromosome Numbers in Plumbaginaceae . . . . .	73-76
Sugiura, T., Studies on the Chromosome Numbers in Higher Plants. III. . . . .	205-212
Sugiura, T., Studies on the Chromosome Numbers in Higher Plants. IV. . . . .	424-333
Sugiura, T., Studies on the Chromosome Numbers in Higher Plants. V. . . . .	363-370
Sugiura, T., Chromosomes Studies on Papaveraceae with Special Reference to the Phylogeny . . . . .	558-576
Suzuki, K., Regeneration of Gonads in <i>Plecoglossus altivelis</i> after Spawning Season . . . . .	113-126
Takamine, N., On the Plasmolysis Form in <i>Allium cepa</i> with Special Reference to the Influence of Potassium Ion upon It . . . . .	302-323
Takamine, N., Studies on the Influence of Ultraviolet Rays on the Cytoplasm, Tested by the Plasmolysis-Form Method . . . . .	577-587
Tanaka, N., Chromosome Studies in Cyperaceae, IV. Chromosome number of <i>Carex</i> species . . . . .	51-58
Tanaka, N., Chromosome Studies in Cyperaceae, VI. Pollen development and additional evidence for the compound chromosome in <i>Scirpus lacustris</i> L. . . . .	348-362
Wada, B., Experimentelle Untersuchungen lebender Zellen in der Teilung. IV. Die Einwirkung des Dampfgemisches von Ammonia und Chloroform auf die Mitose bei den <i>Tradescantia</i> -Haarzellen. . . . .	158-179
Yamaha, G. und Suita, N., Über die Wirkung verschiedener Pufferlösungen auf die <i>Spirogyra</i> -zellen . . . . .	371-381
Yasui, K., Karyological Studies on <i>Iris japonica</i> Thunb. and Its Allies. . . . .	180-188
Yasui, K., Cytogenetic Studies in Artificially Raised Interspecific Hybrids of <i>Pavaver</i> . IX. On the bivalents-association in the meiosis of the PMC of <i>Papaver somniferum</i> . . . . .	551-557

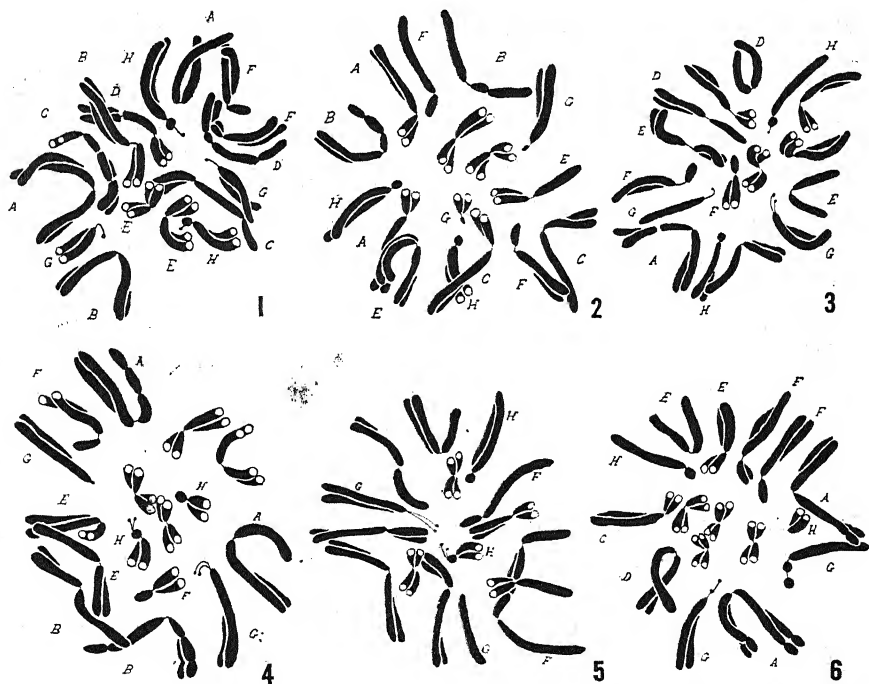
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generally possess a satellite. In the last named type, H, the terminal segment is quite pronounced, at least of the length of the chromosome width or even more, and in most species this segment is connected with a satellite. In all species the F-type appeared to be of the same form, with exception of minor length variations. Secondary constrictions could be observed in the different chromosome-types. They were, however, not well marked enough and could not be placed with such an accuracy that it had allowed their use for an exact chromosome analysis.

A somatic chromosome plate of *C. viticella* is given in fig. 1. The primary constriction is almost median in the chromosome-types A, B and E, whereas in the chromosomes of C and D types it is more submedian. The satellites belonging to the chromosomes G and H are connected with a relatively long thread. The idiogram in fig. 7 shows the average length of the chromosome-types and the position of the primary constrictions (comp. also Table 1). *C. integrifolia* differs from the foregoing species especially through the form of its G and H chromosomes. The satellite of the G chromosome is bigger



Figs. 1-6. Somatic metaphase plates from root-tips. Fig. 1. *C. viticella*. Fig. 2. *C. integrifolia*. Fig. 3. *C. eriostemon* (*C. viticella* × *integrifolia*). Fig. 4. *C. jackmani* (*C. vitalba* × *lanuginosa*). Observe the H chromosome which is here exceptionally provided with a satellite. Fig. 5. *C. Ville de Lyon*. Fig. 6. *C. Prins Hendrik*. The G chromosomes both show tandem-satellites of different outlook. ×2,300.

Table 1. The average length of chromosomes in microns of six *Clematis* species. The G- and H-types are measured without the satellite.

Chromosome-types:	A	B	C	D
<i>C. integrifolia</i>	4.0+5.5=9.5	3.6+4.4=8.0	2.5+5.0=7.5	3.2+4.6=7.8
<i>C. viticella</i>	4.4+5.0=9.4	3.8+4.0=7.8	2.5+5.0=7.5	2.1+4.6=6.7
<i>C. eriostemon</i>	4.0+4.2=8.2	3.4+3.8=7.2	2.5+4.2=6.7	2.7+3.4=6.1
<i>C. jackmani</i>	4.4+4.8=9.2	3.6+4.6=8.2	3.4+5.5=8.9	2.9+4.6=7.5
<i>C. recta</i>	2.9+3.8=6.7	2.7+3.2=5.9	1.9+3.6=5.5	2.1+2.9=5.0
<i>C. Prins Hendrik</i>	3.8+5.5=9.3	4.2+4.6=8.8	2.5+4.8=7.3	3.8+4.4=8.2

Chromosome-types:	E	F	G	H
<i>C. integrifolia</i>	2.5+3.8=6.3	1.3+5.5=6.8	5.3	0.4+5.3=5.7
<i>C. viticella</i>	2.7+3.4=6.1	1.5+5.0=6.5	5.3	0.6+5.0=5.6
<i>C. eriostemon</i>	2.5+2.9=5.4	1.3+4.2=5.5	4.6	0.4+4.6=5.0
<i>C. jackmani</i>	2.1+4.2=6.3	1.5+5.0=6.5	5.3	0.4+3.6=4.0
<i>C. recta</i>	2.5+2.7=5.2	0.8+3.6=4.4	3.6	0.6+5.3=5.9
<i>C. Prins Hendrik</i>	2.9+3.2=6.1	1.3+4.4=5.7	{ 4.2 4.2	0.4+3.8=4.2
				0.6+4.6=5.2

and the connecting thread shorter than was the case in *C. viticella*. The H chromosome in its turn has no satellite at all (fig. 2). In the hybrid between these species, *C. eriostemon*, (fig. 3) accordingly one H chromosome of *C. viticella*-type with satellite and another H chromosome of the *C. integrifolia*-type are easily to be found (in the fig. at 1, and at 6 o'clock). All other chromosome types are too similar in both parent species to be recognized in the hybrid. In the same way in *C. jackmani*, a hybrid between *C. viticella* and *C. lanuginosa*, the six first chromosome-types are quite identical with

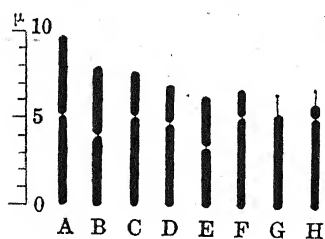
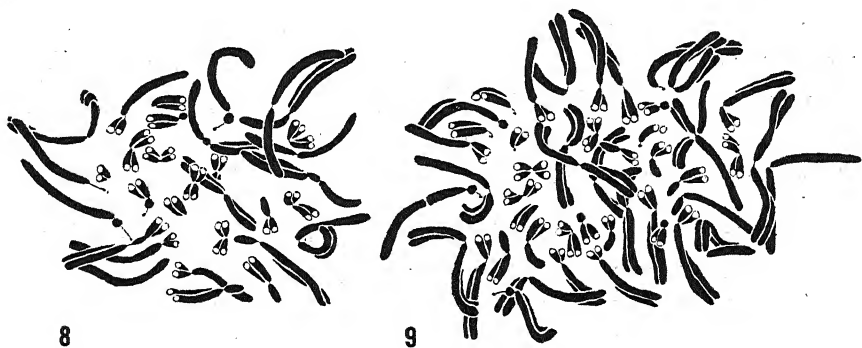


Fig. 7. A diagram representing the somatic chromosome set of *C. viticella*.  $\times 2,300$ .

the corresponding ones in the species already described. On the other hand the G chromosome pair is heteromorphic, one of them is a SAT-chromosome and obviously it thus comes from the *C. viticella* parent, the other lacks the satellite (fig. 4). The two H chromosomes however, are generally rid of satellites contrary to the case in *C. viticella*. Only in one exceptional plate a distinct satellite in one of the H chromosomes was observed (fig. 4). To the *C. jackmani*-group belongs also the garden-form *C. Ville de Lyon*. There again the G and H pairs are both heteromorphic (fig. 5). Another garden-hybrid *C. Prins Herdrik* possesses quite interesting G chromosomes with different sized tandem-satellites and in addition the H chromosomes reveal their dissimilarity, one of them being more subter-

minally constricted than the other (fig. 6). It may be possible that in this species a fragment is present, at least in several plates a small chromatic body appeared. In one case one of the E chromosomes was found as two separate pieces and it looked as if the break had taken place at the primary constriction point. About the cause and significance of such a somatic chromosome breakage nothing more can be said, especially as no analogous findings are known to us.

The tetraploid *C. mandschurica* contains, as is to be expected, 20 V-formed chromosomes, 4 of the F-type and the same number of G- and H- types. Both last named types are apparently similar to those found in *C. viticella*, although the satellites are difficult to distinguish in the whole theoretical number in all plates. The four easily recognizable F chromosomes are to be seen in the lower right-hand- corner of the metaphase plate reproduced (fig. 8). Similarly a number of G and H chromosomes are situated on the opposite side in the same plate. Since the chromosome complements of the different *Clematis* species resemble each other in so a high degree, it has not been possible to conclude with the aid of chromosome morphology, if this species should be considered as an auto- or an allopolyploid. During the reduction divisions multivalent configurations appeared no more than other irregularities. The probability of its being an allotetraploid is thus nearest at hand.



Figs. 8-9. Somatic metaphase plates from root-tips. Fig. 8. *C. mandschurica*,  $2n=32$ .  
Fig. 9. *C. paniculata*,  $2n=48$ .  $\times 2,300$ .

Langlet ('27) has given the diploid chromosome number,  $2n = 16$ , for *C. paniculata*. In our material however, *C. paniculata* originally received from the Hortic. Institution at Alnarp (Sweden), and the name of which without doubt was the right one, showed to be a hexaploid one,  $2n = 48$ . Accordingly we find the corresponding amount of chromosome-types, viz., 30 of the V-types and 6 of each of those belonging to the types F, G, and H (fig. 9). Most probably

all chromosomes of the two last named types are satellite bearing ones.

### Observations from the Reduction Divisions

During the reduction divisions in ten of the *Clematis* species studied a varying amount of irregularities could be seen. The most conspicuous feature was the frequent appearance of chromatid bridges and fragments at the anaphases of both divisions. The origin of such bridges and fragments was first cytologically analysed by McClintock ('33) in maize. According to her the plant studied was an inversion heterozygote, and a crossing-over in the inversion region had resulted in a formation of dicentric and acentric chromatids i.e. chromatid bridges and fragments at the anaphases of the reduction divisions. Later the theory has been developed further by several investigators (Smith, '35; Richardson, '36; Frankel, '37; Darlington, '37; Upcott, '37; Philp, '38; a.o.). Both the number of chiasmata in the inversion itself and those proximal to it, influence the occurrence of such configurations.

Structural hybridity has been found very rarely in the family *Ranunculaceae*. With exception of a few *Paeonia* species (Hicks and Stebbins, '34; Dark, '36; Sax, '37) in none of the other genera anything has been observed which would indicate structural changes in their chromosome complements. On the other hand, of the *Clematis* species here studied the following ones were structural hybrids: *C. integrifolia*, *C. texensis*, *C. fusca*, *C. heracleifolia* var. *dauidiana*, *C. viticella*, *C. lasiantha*, *C. vitalba*, *C. jackmani*, *C. Ville de Lyon* and *C. Hybrida*. The dyscentric changes were most characteristic during the anaphase where the previous crossing-over in the inversion region revealed itself, as general, in the formation of dicentric and acentric chromatids. Of their form in *Clematis* it is impossible to decide in which special chromosome pair and where the inversion in every case had taken place. This is partly due to the uniformity of the *Clematis* chromosomes and may partly depend on the fixation. In every species there naturally must be at least as many inversions as the maximal number of first division bridges per cell indicates.

In *C. viticella* about 13% of the first division anaphases contained bridges, but only one in every cell. Of the eight chromosome pairs in *C. Ville de Lyon* at least five must contain an inversion (fig. 10). Here the number of bridges at the first division anaphases amounted to about 64%. Again in *C. jackmani* only relatively few bridges were present. In about 200 first division anaphases studied, only three cells with one chromatid bridge were found (fig. 11). This may depend of the fact that a relatively high number of chromosomes



normally will be found unconjugated. Therefore configurations indicating inversions may appear in a smaller number than their actual amount would require.

Mostly the bridges break already during the first division as in the fig. 10. Two of the bridges are here thus broken and also in others a thinner region forestalls apparently the later point of breakage. Only in rare cases the bridge may persist and still be present at the second division. The acentric fragments are mostly left in the plasma and will later on most probably be absorbed. Sometimes however, they will be seen still at the second division.

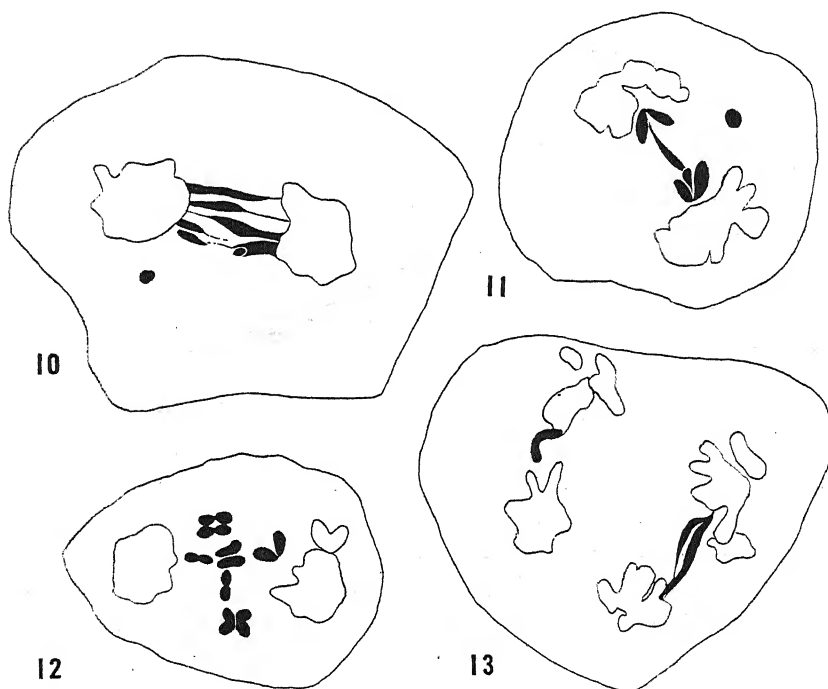


Fig. 10. *C. Ville de Lyon*, first division anaphase in which five bridges and two fragments are visible. Fig. 11. *C. jakmani*, first division anaphase showing a bridge and a fragment. Fig. 12. *C. jackmani*, first division anaphase with lagging univalents, one divided and three split ones at the equator, and one half bivalent nearer to the lower pole. Fig. 13. *C. Ville de Lyon*, second anaphase. In the one spindle there is a double bridge and in the other a fragment still persisting from the first division.  $\times 2,300$ .

In most of the *Clematis* species studied a rather high number of univalents is to be found. This feature is characteristic for structural hybrids, while long inversions especially may easily prevent chromosome conjugation (comp. Frankel, '37). The univalents divide generally during the first division but are retarded in their movements compared with the bivalents. At the late anaphase lag-

ging univalents therefore are frequently met in the neighbourhood of the equatorial plate (fig. 12). The number of univalents is much higher in *C. jackmani* than in others, evidently depending on the nonhomology of its chromosome sets. Fig. 12 illustrates one cell where three divided and one already split univalent and a lagging bivalent chromosome are present. The split and divided univalents generally will not reach the daughter nuclei in time. They are then left in the plasma where they may form small nuclei of their own and later on microcytes. During the second division the already divided univalents do not split another time.

Also in the second division spindles several species show chromatid bridges. In species with pronounced structural hybridity, as for instance *C. Ville de Lyon*, the second division is very irregular. In many cells it looks almost as if the chromosomes would be scattered without any order at all throughout the cell. In the figure reproduced (fig. 13) a rather rare second division double bridge is pictured. Evidently it has arisen through the simultaneous occurrence of two complementary chiasmata in the inversion and one disparate chiasma proximal to it. In the left hand spindle the dark body represents the acentric fragment. The tetrads are in consequence mostly abnormal, containing more or less than four cells.

Due to the irregularity of the reduction divisions the pollen of the different *Clematis* species is more or less poor. The pollen of *C. jackmani* and of *C. Ville de Lyon* is morphologically rudimentary and does not germinate. The pollen of the other species here treated with appears to be good, with the exception of a few misformed cells. The following germination percentages were obtained: *C. viticella* 25%, *C. fusca* 64%, and *C. integrifolia* 10%. The pollen of *C. heracleifolia* var. *daurica* did not germinate at all, although its outlook was relatively normal.

### Discussion

On the ground of its basic chromosome number,  $n = 8$ , the genus *Clematis* is a typical representative of the family *Ranunculaceae*. It seems however, that polyploidy is less common, at least according to this investigation, than in the other genera of the family. Only in the group *Rectae* two polyploid species, *C. mandschurica* and *C. paniculata* have been found. The analysed somatic chromosome complements of 22 different species, horticultural forms and hybrids, belonging to eight different under groups, are surprisingly uniform. Compared with the genus *Ranunculus*, where two morphologically so closely related species as for instance *R. lapponicus* and *R.*

*pygmaeus* reveal quite differently built chromosome sets (Flovik, '36), it is the more apparent.

The form and size of the chromosomes are to be held as much more important taxonomic characters than their number (Langlet, '32). If the karyotypes of two families or species closely resemble each other, we are justified to consider them systematically more related than the other characteristics would allow and vice versa. It should, however, be remembered that these characters, as well as all other ones, "—in verschiedenen Fällen und an verschiedenen Stellen des Systems von verschiedener Tragweite sind, wobei sie immer im Zusammenhang mit anderen systematischen Kriterien angewandt werden müssen" (Lewitsky und Tron, '30, p. 773). A theory has been put forth by Babcock, Stebbins and Jenkins ('37), that inside a plant family the more primitive species belonging even to different genera often resemble in regard to the form and size of their chromosomes each other in a high degree. Contrary to that the more advanced forms show by comparing their chromosome complements dissimilarity also inside one and the same subgenus. The findings in the genus *Clematis* are quite opposed to this view. It is to be remembered that this genus is considered as one of the most developed in the family (Prantl, '88), and yet the chromosome complements in the different species are very uniform. The changes in karyotypes are according to Sharp ('34) assumed to result from fragmentations and translocations. He observes, that in originally unlike chromosome complements such evolutionary changes may reduce this dissimilarity. It implies that "—the same general karyotype may characterize species which are morphologically very unlike" (Sharp, l.c. p. 131). It seems, however, very unlikely that the aforesaid changes could produce so close a resemblance as we meet between the chromosome complements inside the genus *Clematis*. Further the karyotypes reveal here no parallelism with the systematic classification. Species belonging to quite different groups may have very similar sets, whereas in nearly related forms the chromosomes of G- and H-types may differ. In this connection it may be emphasized that *C. alpina* in respect to its chromosome morphology clearly belongs to the genus *Clematis*, so that there seems to be no reason to separate it to a genus of its own, i.e. *Atragene*.

More than two hundred representatives of the family *Ranunculaceae* have been studied by Langlet ('32) with the purpose to investigate the relationship between the taxonomy and cytology in this family. According to his opinion there exist indeed such a state of things. Two different karyotypes could thus be distinguished, the R (anunculus)-type and the T (alictrum)-type, every genus in its

entirety representing either of these. The R-type species were characterized by long, wound or strongly bent chromosomes, the T-type in its turn possesses relatively small and less bent chromosomes. Obviously the *Clematis* species belong to the R-type where they also are counted by Langlet (l.c.). His supposition, however, that the basic chromosome number in this genus would be four, cannot be valid. There is namely no possibility to arrange the different chromosome-types into four groups so that they at least in their general outlook would be comparable.

In all *Clematis* species studied, at least one pair of satellite bearing chromosomes are present. The connecting thread seems to be the only visible constriction in the G chromosomes of *C. viticella*-type. It would be thus the primary one. The primary constriction cannot, however, be identical with the satellite tread according to Delaunay ('29). In this case also we have to assume, that the thread is connected to a short chromosome segment, which, however, is so small that it is practically invisible. The same situation is met with most probably in the G chromosomes of *C. jackmani* and *C. Ville de Lyon*, and in the H chromosome of *C. campaniflora*. The seemingly terminal constriction here evidently must be utmost sub-terminal (Darlington, '37). The tandem-satellites discovered in *C. Prins Hendrik* are of rare occurrence. Previously such ones have been observed in *Aucuba* (Meurman, '29) and in *Paris japonica* (Haga, '34).

In several instances there have been found heteromorphic chromosome pairs in *Clematis*. We refer to the G pair of *C. jackmani*, *C. Ville de Lyon* and *C. Prins Hendrik* and to the H pair of the two last named ones, *C. eriostemon* and *C. campaniflora*. This phenomenon is held by Lesley and Lesley ('35) as especially characteristic for the satellite chromosomes. In fact heteromorphy has been described more commonly in this type of chromosomes than in others (comp. Okuno, '37 in *Lobelia*; Satô, '37 in *Galanthus*, a.s.o.). Still it could be thought that this would only depend on the fact, that already minor changes in the size of the satellites are more easily detectable than similar small diversities in other chromosome segments. The question is further closely combined with the theory of amphiplasty (Navashin, '27; Babcock and Navashin, '30). By crossing *Crepis* species Navashin (l.c.) observed in the hybrids, that in several cases the satellite of a certain chromosome had disappeared. This phenomenon was quite characteristic and constantly observed. It occurred always in the same chromosome in analogous crosses but in other hybrids in different chromosomes. In plants where amphiplasty had been established not one normal plate could be found not

even by the most careful investigation. Apparently this explanation could be considered as probable also in the case of *C. jackmani*, where the satellite had disappeared from one of the parental H chromosomes. It may be stressed, however, that in one metaphase plate (fig. 4) a distinct satellite was present in the H chromosome in question. Here we thus should meet a case of "—eine Restitution der normalen Structur—" (Navashin, l.c.), a situation so far never observed in *Crepis*. The aforesaid satellite in the H chromosome could not have escaped discovery in the other plates studied, as a special attention was paid to it and a number of good plates were available in the material.

The relation between the structural changes and the fertility of the plants is most interesting. The question has been dealt with in some length by Upcott ('37). She has pointed out that the amount of structural hybridity and fertility of the pollen are in a reversed relation to each other in tulips. Plants showing a high amount of structural changes could therefore be propagated only asexually as clones. The author says (l.c. p. 393): "Thus we find throughout the genus, in diploids, triploids, and tetraploids, that the presence of a large number of structural differences is associated with sterility, and may perhaps conclude that this sterility, determined in the first instance by structural differences, itself favours their further accumulation."

In *Clematis* species studied in this respect we find quite the same relation, which is to be seen in the table below:

Table 2. The percentage of chromatid bridges and fertility of pollen etc. in certain *Clematis* species.

Name	Bridges in first division in %	Maximal number of bridges in one cell	The % of germinated pollen	Seed formation
<i>C. Ville de Lyon</i>	64	5	0	none
<i>C. integrifolia</i>	54	3	10	weak
<i>C. fusca</i>	16	1	64	moderate
<i>C. viticella</i>	13	1	25	abundant
<i>C. tangutica</i>	0	0	50	very good

Roughly spoken it can be said that the number of observed bridges and the fertility also here are dependent of each other. Such structural hybrids which at the same time are species hybrids as *C. jackmani* and *C. Ville de Lyon* are the most sterile ones. They are well comparable with the tulips also in that respect that they are propagated only vegetatively. In the obviously allotetraploid *C.*

*mandschurica* in accordance to the allopolyploid tulip species the reduction division is normal and the species quite fertile. The other *Clematis* species stand in their relative fertility between these extremes.

In the foregoing the investigation by Polívková ('36) of the reduction divisions in the P.M.C. and E.M.C. of *C. jackmani* has been mentioned. In this study a special attention is given to the chiasmata and the crossing-over phenomenon. She declares that the bivalents gain during the conjugation a typical form which is characteristic and constant for every one of them. Further, of the eight bivalent present only five show crossing-over. Although our study of the reduction division in *Clematis* is made chiefly with other subjects in mind, we wish to make certain critical remarks against her statements. So for instance in our material the form and build up of the bivalents at the metaphase is not a constant one. The opinion of the author, that there should be special genetical factors which would restrict the crossing-overs to quite definite places in the chromosomes seems not to be founded upon real facts. In *C. jackmani* it cannot be a question of localised chiasmata in the sense of that f.i. stated in *Oenothera* (Darlington, '37). That three bivalents would be formed without any crossing-over at all is equally improbable. The generally accepted view is namely that the whole chromosome conjugation just depends of the crossing-over in the chiasmata (Darlington, l.c.). It may also be pointed out, that the figures reproduced are not convincing enough to admit so far reaching conclusions. Curiously enough Polívková has not observed anything of the very pronounced irregularities which prevail in the reduction divisions of *C. jackmani*. In fact, the occurrence of bridges in a few P.M.C. indicates that the crossing-over here has taken place in the inversion region, whereas in the majority of cases it has occurred in other regions. Already this proves that the crossing-over does not always happen in the same prefixed place in the chromosomes of *Clematis*.

### Summary

In the present study the chromosome number of 25 *Clematis* species, hybrids and horticultural forms is given. One of them was tetraploid,  $2n = 32$ , one hexaploid,  $2n = 48$ , and the rest diploids with  $2n = 16$ .

The karyotypes of twenty-two of these *Clematis* forms were analysed more or less in detail, and it was found that the diploid chromosome complements were in every case very uniform. In all of them the following chromosome-types could be distinguished: five

pairs of V-formed chromosomes viz. A, B, C, D, and E, the subterminally constricted F pair and the G and H pairs with an almost terminal constriction. The two lastnamed ones being generally provided with a satellite. In the tetraploid *C. mandschurica* and the hexaploid *C. paniculata* a corresponding number of different types was present.

In five plants the satellite chromosomes were heteromorphic, and indication of amphiplasty was observed.

Ten of the *Clematis* forms studied were inversion heterozygotes in respect to one or more inversions. In consequence hereof during the reduction divisions chromatid bridges, fragments and other irregularities, as for instance univalents and lagging chromosomes, appeared.

A special attention was paid to the relation between the structural changes and the fertility of the plant. It could be stated, that in forms with a pronounced structural hybridity the pollen was bad and that these forms therefore could be propagated only asexually. The amount of such chromosomal alterations and the grade of fertility are in *Clematis*, comparably to that in tulips, in a reversed relationship to each other.

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**Inversion of a Centric Segment in a Chromosome of  
*Ichthyophis glutinosus* (Linn)**

By

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*Received May 16, 1939*

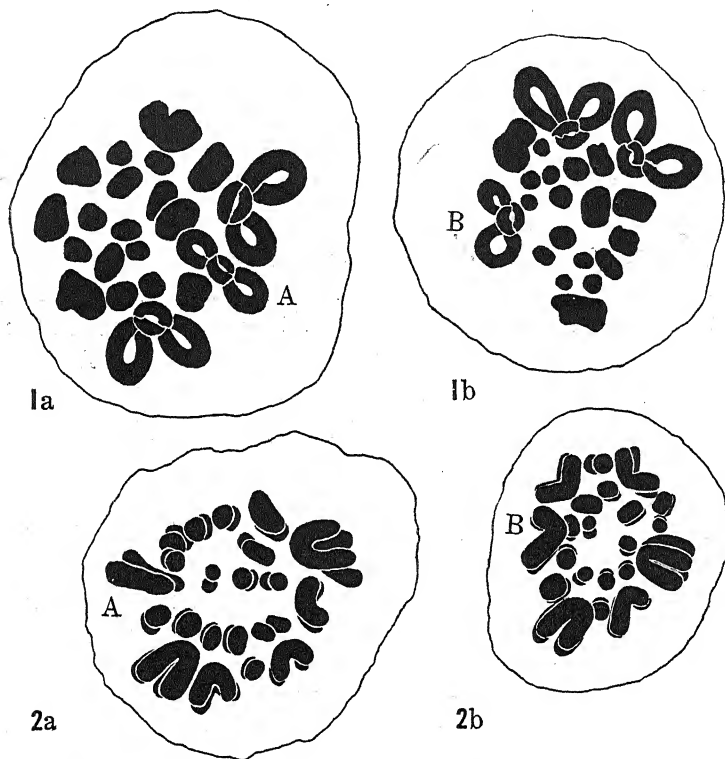
In the course of my examination of sections of the testis of *Ichthyophis glutinosus* I came across a series which showed a change in their chromosome constitution. This variation was observed in all the nuclei and in all stages of spermatogenesis. As it is the first time such a variation was noticed in the chromosomes of *Ichthyophis*, I venture to present it in this short paper.

I have already reported (Seshachar, 1937a, 1937b) that the diploid chromosome number in *Ichthyophis glutinosus* is 42 and that of these, six chromosomes are large and form a conspicuous group on the metaphase plate of a spermatogonium. Four of these six are V-shaped in polar view and are almost identical. They form, during meiosis, two bivalents of large size with four chiasmata in each, in the final stage of condensation. The other two are long rods and they also form a compound bivalent in meiosis with four chiasmata. These three bivalents, two V-shaped and one rod-shaped, are very conspicuous and are most easily recognised in a polar view of the first metaphase plate (Fig. 1a). Their behaviour in meiosis is described in my paper on the subject (Seshachar, 1937b).

I observed during my examination of sections of the testis of this animal, one series, where, in the meiotic metaphase plate, a polar view disclosed the presence of three V-shaped bivalents instead of two V-shaped and one rod-shaped ones (Fig. 1b). On close examination it was noticed that:

- a. The number of bivalents had not undergone any change, i.e., it remained 21, the normal number.
- b. The change had affected only one of the large bivalents, i.e., the rod-shaped bivalent of the normal picture had become a V-shaped one.
- c. No change in size was observed so far as this or any other bivalent was concerned; only, the centromere, instead of being near one end, was now near the middle and was slightly submedian; the bivalent had unequal limbs.

Further, it was noticed that the polar views of Metaphase II revealed the same change. There was no rod-shaped chromosome seen on the normal plate but there was instead a V-shaped one with unequal limbs (Figs. 2a and 2b).



Figs. 1-2. *Ichthyophis glutinosus*. 1, polar view of first metaphase plate.  $\times 3200$ . 1a, a normal individual. A, rod-shaped bivalent. 1b, the abnormal individual. B, the changed bivalent. 2, polar view of second metaphase plate.  $\times 3200$ . 2a, a normal individual. 2b, the abnormal individual.

It was also observed that the change in regard to this chromosome was present in spermatogonia (both primary and secondary) and also in somatic cells (epithelial cells of the testis) where the large rod-shaped chromosomes were replaced by V-shaped ones.

All the above facts pointed to the conclusion that the change affecting the chromosomes of a pair (in a diploid group) was universally found in all the cells of the individual. The testis of *Ichthyophis* is in the form of a number of distinct lobes and the variation was observed in the cells of all the lobes.

It is clear therefore, that this individual is abnormal so far as its chromosome constitution is concerned and also that it is homozygous for the change. It follows that this change occurred a number

of generations back and a distinct population of *Ichthyophis* exhibiting this abnormality must therefore exist. I am unable to confirm this latter possibility on account of the fact that the animals were taken from Kottigehar, in the forest tracts of Mysore State, nearly 200 miles from Bangalore and were collected some years ago. When future collections are made from the area, I shall be in a position to report the correctness of the surmise.

The nature of the change itself can be assumed to be one of inversion of the centromere bearing segment of the AB. It is as shown below:

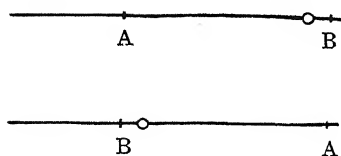
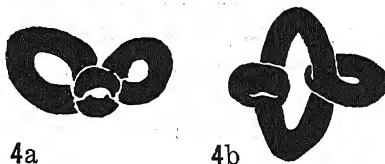


Fig. 3. Diagram to show the method of inversion of the centric segment.



Figs. 4a & 4b. Polar and side views of the changed bivalent at metaphase.  $\times 5600$ .

The change is also such that the centromere in the changed chromosome comes to be placed near the middle of the chromosome resulting in its assuming the shape of a V with unequal limbs.

In normal individuals during meiosis, the bivalent formed from the two rod-shaped chromosomes is such that it forms a series of three rings with four chiasmata of which two are terminal and two interstitial. The centromeres are placed on one of the terminal rings. The inversion of the centric segment has resulted in the formation of a V-shaped bivalent with unequal limbs and where the centromeres are placed on the middle ring.

If the above surmise proves correct, I believe this is the first instance where such a chromosomal change has been observed in a natural population.

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## Studies of Protoplasmic Structure in *Spirogyra*. VI Effects of Sound and Electricity on Elasticity<sup>1)</sup>

By

Henry T. Northen and Robert MacVicar

Received January 7, 1939

Previous investigations from this laboratory have demonstrated that protoplasm in cells of *Spirogyra* is an elastic fluid and probably has a net-like ultra-microscopic structure (Northen 2, Northen and Northen 3). Furthermore, Pfeiffer (7) through investigations on the rate of movement of protoplasts into tubes has also demonstrated that protoplasm is an elastic fluid. All stimulating agents which we have previously investigated, cutting (Northen and Northen 3), anesthetics (Northen 4), heat (Northen 5), uneven pressure (Northen 6), and hypertonic solutions (Northen 6), caused a decrease in the elasticity of protoplasm in cells of *Spirogyra*. The present investigation will demonstrate that sound, direct currents, and alternating currents also cause a decrease in the elasticity of protoplasm. Hence it is hypothesized that stimulating agents initially decrease protoplasmic elasticity probably through loosening the structural network.

### Effect of Sound on Elasticity

**Method.** Three species of *Spirogyra* were used. *Spirogyra 1* was used in experiment one, *Spirogyra 2* in experiment two, and *Spirogyra 3* in experiments three and four. Filaments of *Spirogyra* were placed in a Petri dish and a tuning fork was suspended in the water to a depth of 0.5 cm. In experiment one the filaments were placed about 1 cm. from the fork, whereas in the other experiments they were about 4 cm. from the fork. The fork was then vibrated by striking the part above the water every five seconds. After the filaments had been exposed to the vibrations for the desired time they were laid between two strips of moist cotton and centrifuged with the maximum centrifugal acceleration which did not move the chloroplasts in most cells of control filaments. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced (moved to the centrifugal end) were determined for

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1) Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, No. 172.

experimental and control groups. For each experimental group about 200 filaments were used.

**Data and Discussion.** The data are summarized in table 1.

It will be noted that in all instances the chloroplasts have been displaced in more filaments of those exposed to sound (experimental group) than in those which were not so exposed. The chloroplasts

Table 1. Effect of sound on protoplasmic elasticity

Experiment number	Centrifugal acceleration	Minutes centrifuged	Pitch of fork	Minutes exposed	Percentages of filaments in the cells of which the chloroplasts were displaced	
					In experimental groups	In control groups
1	42.5×G	8	512	8	78%	0%
	42.5×G	8	384	8	88	6
	42.5×G	8	320	8	61	6
2	75.6×G	6	512	8	20%	7%
3	265.5×G	2	512	6	74%	17%
4	1062.5×G	1	512	5	39%	16%
	1062.5×G	1	512	8	47	24

moved more readily in the experimental than in the control groups because the vibrations decreased the value of  $co$  in the equation,  $V = k(c - co)$ , which approximately governs the velocity of chloroplastic movement in response to different centrifugal accelerations, (Northen 2). In the above equation  $V$  is the velocity of chloroplastic movement,  $k$  is a constant,  $c$  is the centrifugal acceleration used, and  $co$  is the initial starting acceleration at which or below which the chloroplasts will not move regardless of how long the filaments are centrifuged. For example, in experiment 1 the chloroplasts were displaced in 78% of the filaments exposed to sound (pitch = 512) and in 0% of the control filaments. At the time that experiment was performed the average value of  $co$  in control filaments was equal to or greater than  $42.5 \times$  gravity. Hence when the control filaments were centrifuged with an acceleration of  $42.5 \times$  gravity the velocity of chloroplastic movement would be:  $V = k(42.5 - 42.5) = 0$  or  $V = k(42.5 - > 42.5) = < 0$ . A velocity less than 0 is impossible. In those exposed to sound the vibrations decreased the value of  $co$  and in 78% of the filaments it was decreased to a value less than  $42.5 \times$  gravity. Hence for such filaments the velocity of chloroplastic movement would be:  $V = k(42.5 - < 42.5) = > 0$ . A decrease in  $co$  has been interpreted as a decrease in elasticity and a decrease in elasticity indicates that the protoplasmic network has been loosened.

### Effect of Direct Current on Protoplasmic Elasticity

**Method.** Platinum electrodes connected through an ammeter and a rheostat to a source of current were placed 1 cm. apart on a strip of wet cotton which was partially immersed in water in a Petri dish. Portions of filaments of *Spirogyra* were then placed over the electrodes so that a current of uniform density passed through the filaments. In each experiment about two hundred filaments were used. After the desired intensity of current had flowed for the desired time the filaments were centrifuged with an acceleration of  $170 \times$  gravity for three minutes. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced in experimental and control groups were determined.

**Data and Discussion.** The data are summarized in table 2.

It will be noted that in all instances the chloroplasts have been displaced in more experimental filaments (exposed to current) than in the control filaments. Hence, it is concluded that with the inten-

Table 2. Effect of direct current on protoplasmic elasticity

Milli-amps	Percentages of filaments in the cells of which the chloroplasts were displaced after exposures of										Range of controls
	0 secs.	5 secs.	10 secs.	20 secs.	40 secs.	60 secs.	90 secs.	180 secs.	360 secs.	600 secs.	
0.15	2%	—	—	—	—	—	—	—	—	65%	—
0.25	1	—	—	—	—	—	—	—	—	78	—
0.50	0	—	—	—	23%	12%	—	56%	—	87	—
1.00	5*	—	—	58%	77	63	75%	78	82%	64	0%-10%
2.00	2*	—	84%	84	76	99	—	—	—	—	0%-3%
4.00	4*	—	92	85	82	—	93	—	97%	—	0%-14%
6.00	0	55%	95	81	32	92	59	—	—	—	—

\* Controls were run with each duration. The average percentage displacement for the controls is recorded in column 2, whereas the range of the controls is recorded in the last column on the right.

sities and durations used direct current decreases protoplasmic elasticity in cells of *Spirogyra* and it is believed that a decrease in elasticity results from a loosening of the protoplasmic network.

### Effect of Alternating Currents on Protoplasmic Elasticity

**Method.** The method was essentially the same as that used with the direct current except that a twelve volt alternating current was used.

**Data and Discussion.** The data are summarized in table 3.

Table 3. Effect of alternating currents on protoplasmic elasticity

Milliamps	Percentages of filaments in the cells of which the chloroplasts were displaced after exposures of					
	0 min.	12.0 min.	15.6 min.	21.3 min.	30.6 min.	48.0 min.
1.0	4%	—	—	—	—	26%
1.25	15	—	—	—	29%	—
1.50	4	—	—	49%	—	—
1.75	7	—	63%	—	—	—
2.00	5	74%	—	—	—	—

In the above experiments the Nernst equation (Heilbrunn 1),  $I\sqrt{t} = k$ , was tested and apparently did not hold for the intensities used. In the above equation  $I$  is the intensity,  $t$  is the duration, and  $k$  is a constant. It will be noted that when a current of 2 milliamps was allowed to flow for twelve minutes the chloroplasts were displaced by the centrifugal acceleration in the cells of 74% of the filaments. If the law be valid and if the intensities were above the threshold the chloroplasts should have been displaced in 74% of the filaments which were exposed to intensities of less than 2 milliamps because the durations with such intensities were calculated from the Nernst equation. For example, the calculated duration necessary to get a 74% displacement with a current of 1 milliamper would be:  $1\sqrt{t} = 2\sqrt{12}$ .  $t = (2\sqrt{12})^2 = 48$ . However, it will be noted that the chloroplasts were displaced in only 26% of the filaments which were exposed to a current of one milliamper. Hence, with the intensities used the Nernst equation did not hold.

However, it will be noted that in all instances the chloroplasts were displaced by the centrifugal acceleration in more cells of experimental filaments than in those of control filaments, thus indicating that the alternating current also decreases the elasticity of protoplasm in cells of *Spirogyra*.

### Summary

Vibrations from tuning forks, direct current of intensities from 0.15 milliamps to 6.00 milliamps, and alternating currents of intensities from 1 to 2 milliamps decrease the elasticity of protoplasm in cells of *Spirogyra* as evidenced by the observations that centrifugal accelerations displaced the chloroplasts more readily in cells exposed to the above stimulants than in cells which were not so exposed.

We are deeply grateful to Mr. Jack Gottlieb who was employed on N. Y. A. for aid in obtaining the data.

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## Studies in the Capparidaceae : V. The cytology of *Crataeva religiosa* Forst.

By

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(With 22 figures in the text)

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### Introduction

This is a continuataion of the series of four papers on the morphology and cytology of some important members of the Cap-paridaceae that are commonly found in India (Raghavan, 1937; 1938a; 1938b & 1939). The plants were grown in the University Botanical garden, and the right stages of flower bud development were determined by aceto-carmin examination of the anthers. The plant flowers during the hot season when it is bare of foliage. The perianth was removed and after prefixation in Carnoy's fluid, were fixed in Navashin's fluid. Osmic fixatives invariably proved ineffective. Smears were also taken, the most successful fixative being Belling's modification of Navashin. It was found that as in the flower buds, in smears also a few seconds' immersion in Carnoy proved very effective. Preparations for cytological study were exclusively stained in Newton's Iodine-gentian-violet, staining having had to be prolonged to about three hours.

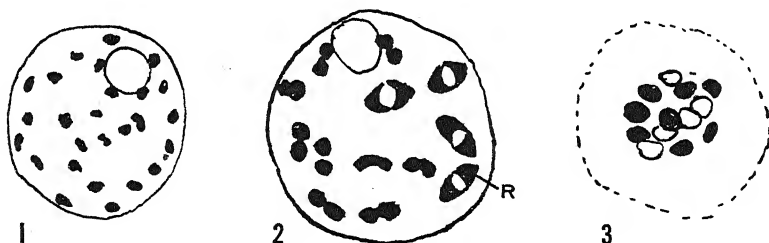
### The Prochromosomes

That there are prochromosomes in the Capparidaceae has already been indicated by their reported occurrence in *Gynandropsis* and *Polanisia* (Raghavan 1938b) and in *Capparis* species (Kuhn 1938 and Schiller 1928). Fig. 1 shows the pollen mother-cell in telophase of the last premeiotic division. Twenty-six prochromosomes, representing the diploid chromosome number of the species, could be readily counted. The occurrence of the full diploid number of prochromosomes in the premeiotic nucleus is directly connected with the question of the presence or not of a leptotene stage in genera exhibiting prochromosome. De Souza (1939) believed that the chromocentres were already paired and that there was no leptotene stage in *Impatiens Balsamina*. On the other hand, Smith (1934) found no evidence for the pairing of the prochromosomes in the

premeiotic nucleus. The occurrence of the diploid number in the premeiotic nucleus of *Crataeva* as in *Gynandropsis* rules out any possibility of the pairing of the prochromosomes at that stage and as such there is likely to be a leptotene stage though possibly of short duration. The threads with the chromocentres at the spindle fibre attachment regions are so very delicate during this stage that it was not possible to count the actual number of these bodies at leptotene, and no detailed study of the meiotic nucleus prior to the onset of diakinesis has therefore been attempted.

### Diakinesis and Later Stages

At diakinesis (Fig. 2) thirteen bivalents are distributed at the periphery of the nucleus. Gates (1909) first observed such a uniform spacing and his suggestion that this was due to a mutual repulsion of the bivalents has been generally accepted. It was further observed that the bivalents are distributed in such a manner that the distances between neighbouring bivalents are fairly regular. Catcheside (1937), who observed a similar phenomenon in *Brassica*,

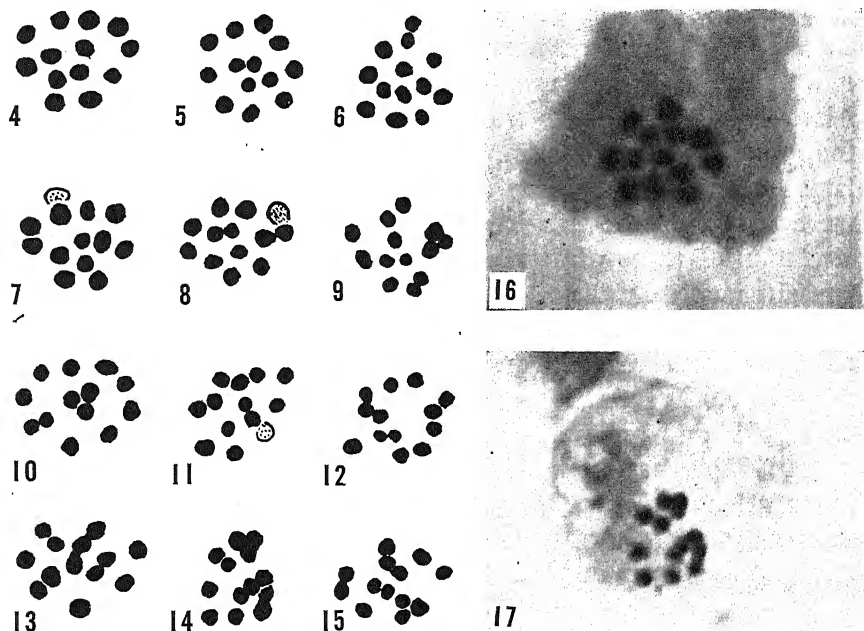


Figs. 1-3. 1. Last telophase of premeiotic pollen mother cell. Note the full diploid number of prochromosomes (26) four of which are attached to the nucleolus.  $\times 3500$ . 2. Diakinesis. Thirteen bivalents are peripherally distributed. Two of these bivalents are attached to the nucleolus. R—ring bivalents.  $\times 3500$ . 3. Prometaphase. The bivalents are clustered together in the centre on account of the loss of repulsion between them  $\times 3500$ .

suggests that there should be an approximately equal force of repulsion between all the bivalents inasmuch as there is an approximately equidistant peripheral arrangement. The nucleolus is also peripheral. No multivalent formation was observed. As diakinesis advances into prometaphase (Fig. 3) the distances between the bivalents diminish, obviously due to the weakening of the repulsive force. This weakening not being regular, there are often one or more groups of secondary paired bivalents. It is suggested that bivalents which are paired secondarily at first metaphase have been neighbours during diakinesis.

Both rod and ring bivalents occur. Of the latter four are usually present (Fig. 2 R), while the rest belong to the rod-shaped kind in which the members of each bivalent are connected by one end only.

At first metaphase the clumping of the bivalents gives place to a gradual spreading apart resulting in a level plate. In the absence of secondary pairing, which is so conspicuous a feature of this stage,



Figs. 4-15.  $\times(3500)$ . Show various degree of secondary association of first metaphase bivalents. Fig. 4 shows no association with nine-four configuration. Fig. 5. Also a nine-four configuration but with a central group of two while the other two central are unassociated. Figs. 14 & 15 show maximum association, groups of three, two of two, and three singles. Fig. 14, same as Text-fig. 17. Fig. 16. First metaphase plate greatly enlarged to show the thirteen bivalents clearly.  $\times$  ca. 2500. Fig. 17. First metaphase showing maximum association of bivalents. Same as Text-fig. 14.  $\times$  ca. 5200.

the thirteen bivalents are usually arranged four in the centre and nine peripherally (Figs. 4 & 5) though eight peripheral and five central arrangement is not infrequent (Fig. 16).

As already indicated first metaphase is characterised by the occurrence of a variable number of bivalents secondarily paired. Figs. 5-15 show various degrees of secondary association. Plates exhibiting no secondary associations are comparatively rare. In such, four central and nine peripheral arrangement is the commonest and as such this has to be regarded as the stable configuration (Fig. 4). Eight-five arrangement is also not infrequent.

With a single secondary pair and eleven free bivalents, a peripheral pair is the commonest (Fig. 6), though occasionally the two bivalents of the secondary pair may be central, leaving two free ones there (Fig. 5). Of the former type the two bivalents of the peripheral secondary pair may be either radial (Fig. 6) or tangential (Fig. 7) in relation to the metaphase plate. With more secondary groupings a distortion of the original unassociated arrangement, eight and five or nine and four, is brought about. But a careful examination of the various patterns exhibited by the metaphase suggests that the central four usually form themselves into two groups of two or sometimes into a central three while the other forms a group of two with a peripheral bivalent (Fig. 10). In one of the plates exhibiting maximum association (Fig. 14), of the central four a group of two has joined a peripheral bivalent to form an association of three while the other two have separately associated themselves with peripheral ones to form a group of three and another of two. In another exhibiting the same groupings (Fig. 15), the two groups of three have presumably been derived by two of the central bivalents forming a group of three with a peripheral bivalent on the left and one joining a group of two towards the right. Evidently this has been derived from an eight-five unassociated configuration, for in addition to these three, which by association with peripheral bivalents have constituted the two groups of three, there is in the centre another group of two.

The accompanying table gives an analysis of the various types of secondary associations met with. The number of secondary pairs per plate ranges from one to six, the mode being three. The maximum association was seen four times. It shows two groups of three, two groups of two and three singles making seven separate associations (Figs. 14 & 15 and Fig. 17).

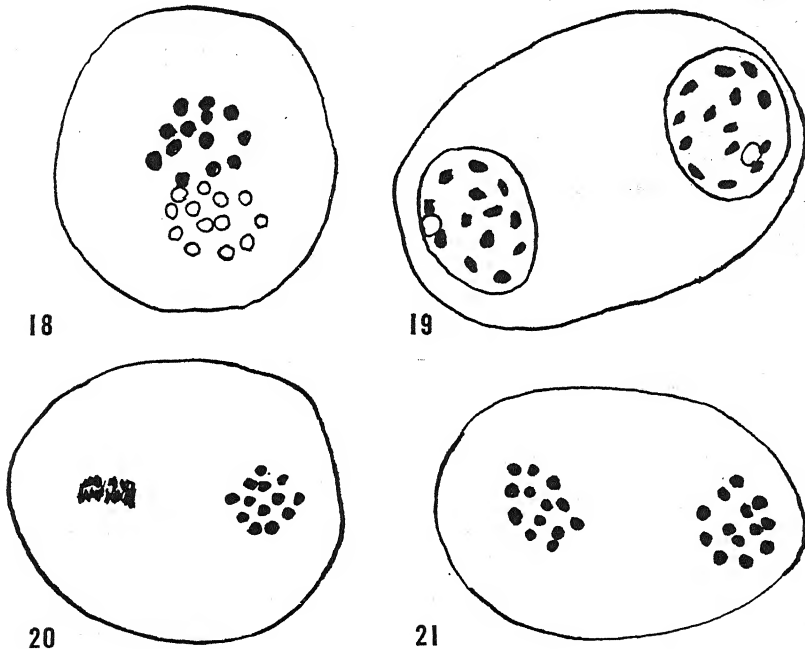
No. of sec. assns.	No. of bivalents in assn.			No. of cases	Total
	1	2	3		
1	11	1	—	4	4
2	9	2	—	5	5
3	8	1	1	8)	15
	7	3	—	7)	
4	6	2	1	8	8
5	4	3	1	5)	8
	5	1	2	3)	
6	3	2	2	4	4
Total					44

The implications and the deductions to be drawn from the pairing of bivalents which are phylogenetically related have already been discussed in connection with the secondary association found in *Gynandropsis*

*pentaphylla* (Raghavan 1938a). In *Crataeva* the maximum number of associations seen four times gives seven groups of chromosomes, two groups of three, two of two, and three singles. Thus the original

ancestor of *Crataeva* would have a basic number seven and *Crataeva religiosa* with  $2n = 26$  is presumably a secondary polyploid.

Fig. 18 shows first anaphase in which the chromosomes in each pole are more or less uniformly spaced. There is very little secondary association exhibited at this stage. In the figure in question there is only one group of two in each pole. Telophase I nuclei are soon built from the anaphase I plates and this passes on to interphase during which there is a further axial expansion of the nucleus. The chromosomes are peripherally distributed. Fig. 19



Figs. 18-21. 18. First anaphase. Only one group of two is seen in each pole; the others are unassociated. (3500). 19. Interphase. Note that two chromosomes are attached to the nucleolus.  $\times 3500$ . 20. Second metaphase, the planes of division of the two plates being at right angles to one another. 21. Do. Both the plates are in the same plane of division. The chromosomes show some amount of accociation.

shows interphase in which the nucleolus is seen with two chromosomes attached to it. Soon the chromosomes approach one another, the nuclear membrane disappears and second metaphase is entered upon (Fig. 21). Both the plates may be in the same plane, as in Fig. 21 where the two plates are under polar view, or they may be at right angles to one another as in Fig. 20. Metaphase II also shows some amount of secondary association. The microspore nuclei that are ultimately organized may be arranged either tetrahedrally or in a quardrite manner.

## The Nucleolar Behaviour

That this is a secondary polyploid derived from a seven chromosomed ancestor—a conclusion reached by maximum association of bivalents at metaphase I—was also corroborated by the nucleolar behaviour. It is now generally admitted that the nucleoli are organized at telophase by definite bodies in satellited chromosomes. These were designated the nucleolar organizing bodies by McClintock (1934) who established this definite relationship. Gates (1937) has given recently a critical survey of papers connected with the discovery of the relation between the nucleolus and the chromosomes. There is no need to cite all the papers connected with this except the important contributions of Heitz (1931 a and b) and De Mol (1926). It is admitted that the number of nucleoli that originate in the telophase, and therefore the number of satellited chromosomes, is a reliable guide to the polyploidy of the plant. For this the work of De Mol (1926) on triploid and tetraploid hyacinths is mainly responsible. He found that the number of nucleoli in the resting nucleus was correlated with the number of monoploid complements present. Often however the nucleoli which originate at telophase fuse together so that in the resting stage we have a single large nucleolus. It follows therefore that the polyploidy of a plant could be inferred from the number of satellited chromosomes, which according to Heitz (1931a) must present in the complements of all plants. Satellited chromosomes, however, cannot always be recognised

owing chiefly to the delicate nature of the chromatin, but the attachment of particular chromosomes to the organizing nucleolus gives the clue in such cases.

In the plant under investigation satellited chromosomes could not be recognised. But the attachment of a definite number of chromosomes at various stages of meiosis gives unmistakable evidence of the polyploidy of the plant. Fig. 22 shows a photomicrograph of the pollen mother cell at diakinesis. The attachment of two bivalents to the nucleolus is unmistakably evident. Not infrequently



Fig. 22. Pollen mother cell in diakinesis with two bivalents attached to the nucleolus. ca.  $\times 5000$ .

two nucleoli are present with one bivalent attached to each. At first telophase and at interphase two chromosomes are always attach-

ed to the nucleolus. These facts go to show that in the monoploid complement of *Crataeva* the organization of the nucleolus is governed by a pair of satellited chromosomes. In other words, in the somatic complement two pairs of Sat-chromosomes must be responsible for the formation of the nucleolus. Fig. 1, which is a premeiotic nucleus, shows the full diploid number of prochromosomes. Four of these bodies are definitely attached to the nucleolus. This means that the plant is a tetraploid though a functioning diploid.

### Discussion

It is thus to be inferred that *Crataeva religiosa* is a secondary polyploid. This is established primarily by the occurrence of secondary associations supported by nucleolar behaviour. The basic number on the criterion of maximum association is found to be seven. But the important thing to be considered is in respect of the occurrence of the same basic number in another genus of the family, *Gynandropsis*. Instances may be quoted where basic chromosome number serves to distinguish between genera of the same family. Thus Chiarugi (1925) found in the Cistaceae that *Cistus* and *Halimium* have the same basic number nine, and *Helianthemum*, the basic number eight. Systematists have included *Halimium* ( $b = 9$ ) in *Helianthemum* ( $b = 8$ ) or rather they have regarded these two genera as more nearly related to each other than either of them to *Cistus*. Stapf (1928) on morphological grounds disagreed with the older systematic position assigned to *Halimium* and regarded it as being more closely allied to *Cistus* — a view corroborated by cytological evidence. Genetical evidence also supports this conception, hybrids being found between *Cistus* and *Halimium* (Warburg O.E. and E.F. 1930). In this instance therefore, chromosome number confirms an affinity suspected on other grounds. It is safe to conclude that within a family the occurrence of the same basic number would imply a close relationship between the genera exhibiting it. On this basis the genus *Gynandropsis* and the genus *Crataeva* would have to be considered as being very closely related. Systematically however they are very far apart in the family, *Gynandropsis* being included within Cleomoideae and *Crataeva*, within the Capparidioideae. In this tropical family Capparidaceae, the criterion of distinction between the two important tribes seems to be the nature of the stem, the Cleomoideae being herbaceous, the Capparidioideae, woody.

Though one is tempted to suggest that this may not altogether be a sound basis of classification, the affinity between these two

genera implied by the basic chromosome number is likely to mean one of two alternatives, either that both these genera are to be placed close together, in which case the division of the family into the said groups would be untenable, or both these genera must have evolved from an ancestor, however remote, whose chromosome number was seven.

It is generally accepted that perennials usually precede annuals in evolution and the suggestion made in the previous paper (1938b) is supported by the observations herein made that seven is likely to be the primary basic number, the chromosome numbers of the different genera and species representing different balances of the same number. The chromosome numbers so far known in the family are so few that it is not possible to discuss the phylogeny of the different genera. But so far as available it would appear that nine must be regarded as the primitive chromosome number of the family, chiefly because this number is the lowest found. The fact that it is a *Capparis* species, *cynophallophora*, lends support to the suggestion made already that from the primary basic-numbered ancestor there arose first the arboreal Capparidoideae and then the herbaceous Cleomoideae. The possibilities of the manner in which the species with higher chromosome numbers have arisen are fragmentation, reduplication of chromosomes by various methods, and hybridization and subsequent reduplication. A critical study of the somatic chromosome complements of the various genera and details of meiosis will throw some light on these. Genetical experiments may also show the presence of multiple factors. Along with these the determination of the chromosome number of the various species will help in the inference of the phylogeny of the various genera of the family. Work along some of these lines, which is being continued will, it is hoped, clear up some of these points in this meagrely worked albeit important tropical family.

### Summary

The haploid chromosome number of *Crataeva religiosa* Forst. is 13. Secondary association of the bivalents has been recorded and the basic number based upon maximum association is seven.

The behaviour of the nucleolus is described, and two chromosomes are found to be responsible for the organization of the nucleolus in the monoploid complement. From both nucleolar behaviour and the occurrence of secondary pairing it is concluded that the plant is a secondarily balanced allotetraploid.

It is further suggested that the number seven is likely to be the primary basic number and that the chromosome numbers of the



various genera represent different balances of this primary number. From this it is suggested that the Capparidioideae first arose, and then the Cleomoideae, by allopolyploidy.

In conclusion Dr. T. S. Raghavan has great pleasure in tendering his grateful thanks to Prof. R. R. Gates F. R. S., for the continued interest evinced in the progress of this work and for much useful criticism.

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**La loi de Robertson et la formule chromosomiale chez deux Lacertiens :  
*Lacerta ocellata* Daud., *Psammodromus hispanicus* Fitz.**

par  
**Robert Matthey**

*Reçu le 16 janvier 1939*

**Introduction**

La famille des *Lacertidae*, dont de nombreux représentants ont été étudiés par K. NAKAMURA, par K. OGUMA et par moi même, présente une remarquable homogénéité cytologique. A l'exception du seul *Lacerta vivipara* dont le nombre diploïde est de 36 chez le mâle, soit 36 bâtonnets de longueur régulièrement décroissante (OGUMA, 34; MATTHEY, 34), toutes les autres espèces examinées possèdent en outre une paire de microchromosomes supplémentaires, ce qui porte le nombre  $2N$  à 38. Ces espèces sont: *Takydromus tachydromoides*, *T. formosanus*, *T. septentrionalis* (NAKAMURA, 28, 31, 35), *Lacerta muralis*, *L. agilis*, *L. viridis* et *Tropidosaurus algirus* (MATTHEY, 29, 31). J'ai admis, dans mes travaux précédents que cette formule s'expliquait de la façon suivante: le nombre fondamental de chromosomes chez les Sauriens étant égal à 48, soit 24 macrochromosomes et 24 microchromosomes, j'ai supposé que ces derniers, unis deux à deux, ne formaient plus que 12 éléments dans les cellules des Lacertidés, dont le *L. vivipara* apparaît alors comme le représentant chromosomialement typique. La paire de m-chromosomes des espèces dont le nombre diploïde est de 38 pourrait résulter, soit d'une néoformation, soit, plus vraisemblablement, de l'absence de fusion pour deux paires microsomiales. Quant aux grands éléments, on se rappellera peut être avec quelle élégance la théorie de ROBERTSON expliquait leur évolution. Je me contenterai de citer ici la belle série de familles que l'on peut relier entre elles grâce à l'application de cette loi:

<i>Agamidae</i> , <i>Iguanidae</i> , etc.	12 V
<i>Helodermatidae</i>	10 V plus 4 I
<i>Varanidae</i>	8 V „ 8 I
<i>Xantusiidae</i>	6 V „ 12 I
<i>Anguidae</i> ( <i>Pseudopus</i> , etc.)	4 V „ 16 I
<i>Anguidae</i> ( <i>Gerrhonotus</i> )	2 V „ 20 I

D'autre part, à l'intérieur d'une famille, la loi de ROBERTSON demeure souvent valable: chez les *Geckonidae*, chez les *Scincidae*,

par exemple. Nous allons, chez les *Lacertidae*, retrouver, une fois de plus, le même mécanisme.

### Matériel et technique

Je remercierai tout d'abord le Professeur E. CHATTON, Directeur du Laboratoire Arago à Banyuls sur Mer (France), qui a bien voulu me faire expédier un beau mâle adulte de Lézard ocellé et plusieurs *Psammodromes*. Les testicules du premier furent prélevés, sous anesthésie à l'éther, le 10 juin et le 2 septembre. Des fragments de ces organes ont été immédiatement fixés selon l'insurpassable méthode de MINOUCHI-NAKAMURA (cf. MATTHEY, 31, 33, 36, 38) par les liquides de CHAMPY et de FLEMMING-HEITZ. Les *Psammodromes* furent simplement décapités et leurs gonades fixées selon la même technique. Les coupes épaisses de 10 à 12 microns, ont été colorées par l'hématoxyline ferrique et par le FEULGEN.

Les dessins ont été effectués avec la combinaison optique suivante: Immersion 1/16 ème de LEITZ et oculaire périplan 25; le grossissement obtenu est de 3800; l'esquisse obtenue est reproduite au double de ses dimensions primitives, puis ramenée à 4100 par la reproduction.

### Observations personnelles

#### A) *Psammodromus hispanicus* Fitz.

Je serai très bref au sujet de cette espèce, chez laquelle on rencontre un comportement chromosomique identique à celui des *Lacertidae* étudiés jusqu'à ce jour. Les figures 1 et 2 montrent en effet que les cinèses goniales renferment 38 chromosomes télomitiques, dont une paire de microchromosomes. Les métaphases auxocytaires contiennent 19 tétrades d'attachement nettement terminal; de ces 19 bivalents, le plus petit correspond évidemment aux deux microéléments observés dans les divisions mitotiques.

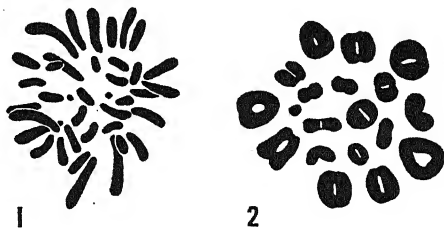


Fig. 1-2. *Psammodromus hispanicus*. 1. Métaphase spermatogoniale. 2. Métaphase auxocyttaire.

En un mot, les conditions sont absolument semblables à celles que NAKAMURA et moi avons relevées chez les *Lacerta*, les *Tropidosaururus* et les *Tachydromus*. Nous allons voir qu'il n'en est pas de même chez le Lézard ocellé.

### B) *Lacerta ocellata* Daud.

Comparé à celui des autres Lézards européens, le testicule de *L. ocellata* frappe par sa richesse en tissu interstitiel; les canalicules séminifères sont littéralement enrobés dans la masse de ce tissu, lequel, chez les *Lacertidae* de faible taille n'est représenté que par des îlots de petites dimensions. J'ai en effet l'impression que c'est le conjonctif intercanaliculaire, plus que les cellules sécrétrices, qui se trouve intéressé par cette hypertrophie, laquelle serait une simple réponse aux problèmes mécaniques posés par des organes volumineux.

**Cinèses goniales:** Les cinèses goniales sont assez peu abondantes, aussi bien dans le matériel élaboré en juin que dans celui fixé en septembre.

La chose n'est pas étonnante, car, chez les autres Lacertidés étudiés, la principale période de multiplication tombe en mars-avril, une deuxième prolifération, moins importante, se manifestant en août. J'ai cependant rencontré une douzaine de figures analysables, sinon parfaites, et dont je figure la plus belle (Fig. 3). Il s'agit d'une métaphase de spermatogonie primaire, montrant, avec une très grande clarté, 36 chromosomes, soit 2 V, 32 bâtonnets de longueur régulièrement décroissante et une paire de m-chromosomes.

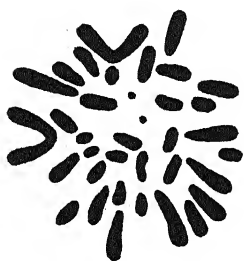


Fig. 3. *Lacerta ocellata*:  
métaphase spermatogoniale.

Dans les spermatogonies secondaires, dont les caryocinèses se rencontrent alors, non pas isolées, mais en petits groupes, les numérations sont plus difficiles, mais les deux V sont toujours bien distincts.

**Cinèses auxocytaires:** J'ai rencontré, en grand nombre, des divisions de ce type au mois de juin; en septembre, elles sont au contraire devenues très rares. Les 18 tétrades sont disposées de la façon habituelle chez les Lacertidés, les géminis les plus grands formant, à la périphérie, une couronne entourant les éléments plus petits. Les grands bivalents revêtent l'aspect d'anneaux équatoriaux, à l'exception du plus grand de tous, tétrade d'attachement médian, laquelle correspond évidemment aux deux chromosomes atélomitiques des cinèses diploïdes. Les géminis centraux, plus petits, apparaissent comme formés chacun de deux bâtonnets parallèles ou légèrement entrecroisés. Enfin, une microtétrade résulte de l'accouplement des deux m-chromosomes observés dans les mitoses spermatogoniales. (Fig. 4-7).

A l'anaphase, il y a partage en deux dyades symétriques de

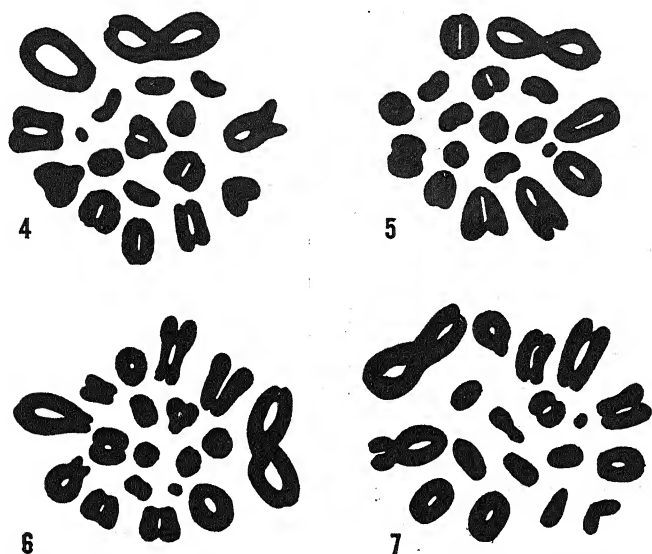


Fig. 4-7. Métaphases auxocytaires. Remarquer les 18 tétrades, dont un volumineux bivalent en V.

chacune des tétrades, ce qui nous indique l'existence probable d'une monogamétie mâle de type X-X.

**Secondes cinèses:** Les figures de division du spermatocyte de deuxième ordre sont abondantes en juin, mais manquent totalement en septembre. Leur difficile analyse permet de retrouver, parmi les 18 dyades qu'elles comptent, une dyade anaschiste en V fissuré et 17 univalents en V simple, ou en courts bâtonnets; la microdyade est en général visible (Fig. 8-11).

De la description de ce cycle chromosomique, nous pouvons maintenant tirer les éléments d'une discussion relative aux mécanismes de l'évolution chromosomique.

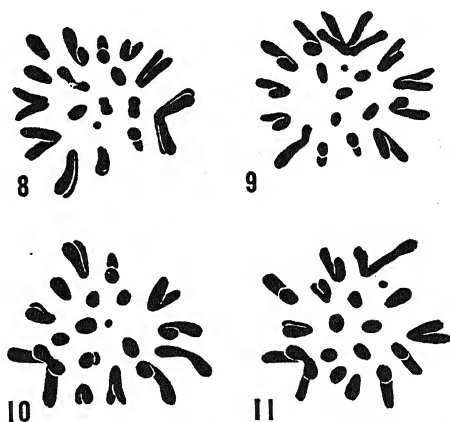


Fig. 8-11. Métaphases de seconde cinèse. Remarquer le V anaschiste unique.

### Discussion générale

Le mécanisme de formation des V par fusion apicale de deux chromosomes télomitiques hétérologues, ne paraît guère, aujourd'hui, rencontrer la faveur des cytologistes: DARLINGTON (37), par exemple,

présente le cas des *Fritillaria* et des Acridiens, mais ne cite pas ROBERTSON et se contente de donner, sous une forme presque dubitative, l'explication d'une évolution chromosomiale par fusion et fragmentation. Dans les dernières pages de son traité de Cytologie, le même DARLINGTON analyse les conditions mécaniques de la fusion et de la fragmentation et parvient ainsi à la conception d'un "inequal interchange" s'éloignant beaucoup, par les résultats qu'il engendre, des idées défendues par ROBERTSON.

L'hypothèse de l'échange inégal n'est guère soutenable que pour des chromosomes dont l'insertion est sub-terminale; un "bras court" est en effet nécessaire pour que soit possible la formation des V. Dans le cas concret qui nous occupe, voici quelles seraient, en partant d'un ancêtre à 36 chromosomes sub-télomitiques, les modifications aboutissant à la formule chromosomiale du Lézard ocellé (Fig. 12).

En A, nous voyons deux paires chromosomiques: entre deux individus, appartenant chacun à l'une de ces paires, un échange se produit; à la suite de celui-ci (B) le nombre n'est pas altéré, mais nous avons maintenant: 1 chromosome en V, un chromosome en I, un m-chromosome et un court fragment acentrique voué à la disparition. Parmi les gamètes issus d'une telle cellule modifiée, il en sera qui renfermeront le V et le micrososome (C, à gauche); un tel gamète, rencontrant un autre gamète provenant d'un individu normal (C, à droite), donnera naissance à une  $F_1$  dont les cellules diploïdes auront

précisément la composition de C. Deux gamètes modifiés, provenant d'individus différents de cette première génération, pourront être à l'origine d'une  $F_2$  (D), possédant cette fois deux V et deux m-chromosomes: la combinaison devenue homozygote se maintiendra par la suite.

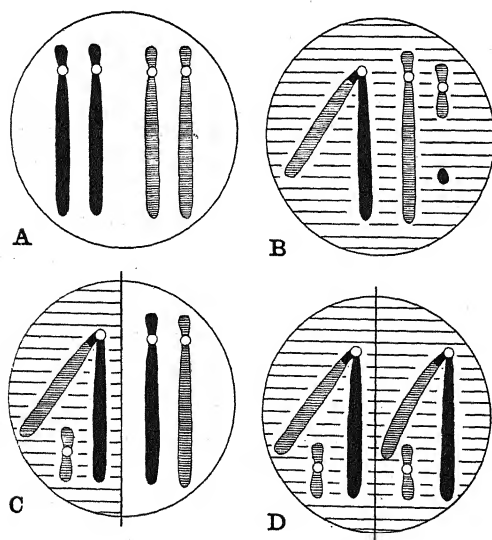


Fig. 12. La formation des V, d'après le mécanisme de l'échange inégal.

Il n'y a rien là qui soit inadmissible: cette explication rend compte à la fois de la formation des V et de celle des microchromosomes. Deux critiques doivent cependant lui être adressées.

1) L'échange inégal n'aboutit à la formation de V que si l'insertion est sub-terminale; chez le Lézard ocellé, je n'ai pu mettre en évidence les centromères; un tel attachement est donc concevable, en dépit du caractère apparemment terminal de l'accrochement. Mais il existe de nombreux animaux dont les chromosomes sont rigoureusement télomitiques (Cf. MATTHEY, 38) et pour l'évolution chromosomiale desquels l'interprétation de DARLINGTON cesse d'être valable.

2) Beaucoup plus grave est la constatation suivante: l'échange inégal ne respecte pas le nombre fondamental: il peut aboutir à des formules où le nombre de chromosomes d'un groupe systématique donné, exprimé en éléments télomitiques, cesse d'être une constante. Or, si la loi de ROBERTSON exige cette constante et l'explique, l'hypothèse de DARLINGTON, au contraire, ne légitime pas son existence. La revue des études de Cytologie comparée (cf. PERROT, 38) démontre que dans la majorité des cas, tout semble s'être passé conformément aux idées de ROBERTSON: un nombre fondamental caractérise bien souvent un groupe donné et il serait bien étonnant que, d'entre les innombrables mutations chromosomiques que peut engendrer l'échange inégal, celles là presque seules soient venues jusqu'à nous, aient été filtrées, chez lesquelles le nombre fondamental se trouvait par hasard conservé.

Il est évident que l'hypothèse de ROBERTSON nous apporte, par contre, une explication simple et immédiate de la formule chromosomiale du Lézard ocellé: les deux V de cet animal correspondent à quatre éléments télomitiques des autres Lacertidés et le nombre fondamental demeure égal à 38. La formation d'un V aux dépens de deux bâtonnets implique les postulats suivants: 1) l'existence d'éléments véritablement télomitiques; 2) l'union au niveau des centromères et la fusion de ceux-ci en un centromère unique, ou la coexistence de ces deux centromères, ou la perte de l'un d'entre eux. Le premier des postulats correspond pleinement à la réalité cytologique, nous pouvons l'admettre sans autre. Le second, avec ses trois éventualités, pose un problème de solution plus délicate.

Le cas des *Gerrhonotus* me paraît susceptible d'éclairer la question: j'ai montré (31, 33) que chez ces Anguidés, on rencontrait des individus ayant, les uns 21 chromosomes dont un V, les autres 20 chromosomes dont deux V. La métaphase auxocytaire montre dans les deux cas 10 tétrades dont une en V. Il est donc certain que cette tétrade peut se former, soit à partir de deux éléments goniaux atélomitiques, soit à partir de trois, l'associé synaptique du V unique étant alors représenté par deux bâtonnets, ayant chacun leur centromère, comme le prouve le comportement absolument normal de ces

chromosomes lors des divisions mitotiques. Un raisonnement de symétrie conduit à admettre que la tétrade atélomitique doit avoir, au total, quatre centromères, et que, par extension, ce nombre doit caractériser les bivalents en V d'origine nettement robertsonienne. Il y a là un point que l'étude d'un matériel favorable, batraciens ou acridiens, permettra peut être d'élucider. En tout cas, le Gerhronote montre sans ambiguïté le caractère composé de son ou de ses chromosomes en V. Pour terminer, je placerai ici, (Fig. 13) les

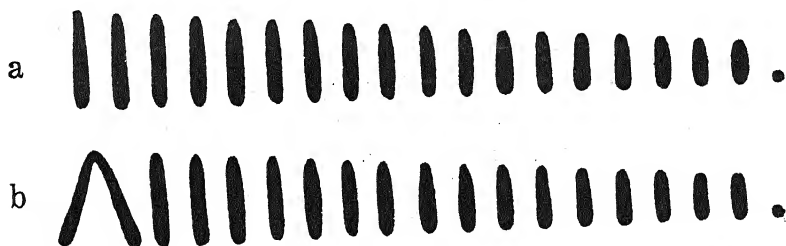


Fig. 13. Schéma représentant l'équipement chromosomique haploïde chez *Psammomodromus hispanicus* (a) et *Lacerta ocellata* (b).

caryogrammes schématiques relatifs aux deux espèces que j'ai étudiées. Ce rapprochement illustre d'une façon qui me paraît bien persuasive, le mécanisme robertsonien de l'évolution chromosomique.

### Conclusions

1) Le Psammodrome espagnol possède, à l'état diploïde, 38 chromosomes, soit 36 éléments télomitiques de longueur régulièrement décroissante et deux m-chromosomes. Les divisions réductionnelles montrent 18 tétrades en bâtonnets et une tétrade punctiforme.

2) Le Lézard ocellé possède à l'état diploïde, 36 chromosomes, soit 32 éléments télomitiques de longueur régulièrement décroissante, deux grands V et deux m-chromosomes. Les divisions réductionnelles montrent 16 tétrades en bâtonnets, une tétrade en V, une tétrade punctiforme.

3) En absence d'hétérochromosomes identifiables, il faut admettre que, chez les deux espèces, le mâle, monogamétique, possède la formule X-X.

4) Cette formule est facile à relier à celle des autres Lacertiens par la théorie de ROBERTSON. Par contre, il est des plus douteux que les faits s'expliquent dans la conception d'un échange inégal.



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## Disturbances in the Process of Cell-Division in the Pupal Wing of the Flour-Moth *Ephestia kühniella* as Result of Heat-Treatment

By

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In 1936 the author published a paper on the mitosis-pattern in the pupal wing of the flour-moth *Ephestia kühniella* (Braun 1936). This paper contains an investigation on the period of cell-divisions in the young pupal wing during which the mitoses are distributed corresponding to the subsequent wing-pattern, and of the role of this stage in the development of the wing-pattern. In one experiment young pupal wings were treated with heat in order to see if the shifting of the subsequent pattern after heat-treatment also shifts the mitosis-pattern. A change in the mitosis-pattern could be observed but in addition the process of the cell-division showed an abnormal behavior after heat-treatment. Brief mention of this fact was made in the original paper. A recent and more extensive calculation of these data on the abnormal cell-divisions is reported in this note.

### Material and Methods

The data for the number of mitoses and the ratio of metaphases to anaphases in the untreated pupal wings of *Ephestia kühniella* were obtained from counts of the number and stages of cell-divisions on individual wings. These wings were removed from pupae which were fixed at different known ages of pupal development. Bouin-Allen solution was used as fixative and the wings were stained with Haematoxylin (Delafield). The cell-divisions were clearly visible on the unsectioned wing and metaphases and anaphases easily distinguishable. The data for the wings from heat-treated pupae were obtained from wings which were fixed 12, 24 and 36 hours after the pupae had been exposed to heat-treatment. The pupae were treated at a pupal age of 54–60 hours, the "sensitive period" for the shifting of pattern elements. They were exposed to a temperature of 44.5°–45.5° for 45 minutes.

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### Observation

The number of cell-divisions and the percentage of metaphases to anaphases for individual wings are represented in tables 1 and 2. The average number of cell-divisions during the entire mitosis-period (40-140 hours) observed on untreated wings is 261. The average metaphase-anaphase ratio is 100:35. The wings from heat-treated pupae however show a great change in the metaphase-anaphase ratio, ranging from 100:416 to 100:127, 12 to 36 hours after the exposure to heat. The average number of cell-divisions in these wings is 158. The great increase in metaphases is observed all over the wing. Wings No. 188 and No. 160<sup>I</sup>, however, fixed 36 hours after heat-

Table 1. Number of cell-divisions, metaphases and anaphases on untreated pupal wings of *Ephestia k.* at different ages.

Number of wing	Age of wing in hours of pupal age	Number of metaphases	Number of anaphases	Metaphases-anaphases ratio
76	48-54	137	89	100 : 65
71	48-54	403	168	100 : 41
70	54-60	364	41	100 : 11
75	54-60	106	36	100 : 34
69	60-66	256	79	100 : 31
65	66-72	250	16	100 : 7
46	72-78	301	115	100 : 38
24	73-79	121	56	100 : 46
57	78-84	343	117	100 : 34
135A	78-84	100	31	100 : 31
113A	84-90	138	65	100 : 47
108A	96-102	144	92	100 : 64
124	108-114	86	48	100 : 56
115B	120-126	27	26	100 : 97
Summa		2776	979	
Average number of cell divisions on each wing:			266	
Average metaphase-anaphase ratio				100 : 35

Table 2. Number of cell-divisions, metaphases and anaphases on pupal wings from heat-treated pupae of *Ephestia k.* at 12, 24 and 36 hours after heat-treatment.

Number of wing	Age of wing in hours of pupal age	Number of metaphases	Number of anaphases	Metaphases-anaphases ratio	
155 <sup>I</sup>	72-78	87	139	100 : 160	Average number of cell-divisions on each wing: 158 Average metaphase-anaphase ratio: 100 : 218
155 <sup>II</sup>	72-78	53	172	100 : 342	
160 <sup>II</sup>	72-78	63	110	100 : 175	
160 <sup>III</sup>	72-78	25	104	100 : 416	
179 <sup>I</sup>	80-90	30	75	100 : 250	
179	84-90	41	52	100 : 127	
160 <sup>I</sup>	96-102	148	61	100 : 41	Average number of cell-divisions on each wing: 215
188	96-102	158	64	100 : 40	Average metaphase-anaphase ratio: 100 : 40

treatment, show a nearly normal ratio of metaphases to anaphases and nearly the same number of cell-divisions as in an untreated wing. The average number of cell-divisions for these two wings is 215. (In addition wings No. 188 and 179<sup>I</sup> showed a clearly shifted mitosis-pattern; No. 155<sup>I</sup> and 160<sup>III</sup>, a slightly shifted mitosis pattern.) Any disturbances in the chromosomes themselves could not be detected in these unsectioned wings.

### Discussion

The effect of extreme environmental conditions on the process of cell-division has been described by many authors but not much statistical material is available for the influence of heat on cell-division. In Pollitzer's book "Pathologie der Mitose" (1934) many experiments on environmental effects on cell-division are reviewed. He reports an arrest of cell-division and slowing down of cell-division after X-radiation and after treatment with chemicals. In the chapter on the influence of heat on cell-division he describes Jolly's, Kohott's, Wassermann's and Kemp and Juul's experiments with plant- and animal-material. They found that abnormal temperatures interrupt cell-divisions and inhibit the occurrences of new cell-divisions. Wassermann furthermore observed that the prophases do not enter the metaphase-stage and that the telophases are slowed down. Kemp and Juul reported an arrest of cell-divisions at the metaphase-stage. Upon exposure to normal conditions the normal process of cell-divisions will reoccur after some time. A delay at the metaphase stage was also found in living cells by Shigenaga (1937) after treatment with chemicals. A decrease in the number of chiasmata in *Vicia faba* after exposure to high temperature was reported by Straub (1937). Marquardt (1937) reviewed the influence of radiation on the process of cell-division in a recent paper. He differentiated three stages: 1. the primary effect, consisting of pathological disturbances of those chromosomes which were in the process of division during the time of radiation; 2. a period free of mitoses and 3. a secondary effect on those cell-division which occur after the mitosis-free period, consisting of translocations, deletions and inversions.

The author's observations show a very great number of anaphases after heat-treatment and therefore suggest, at first glance, an arrest of cell-divisions in the anaphase stage, although arrest at the anaphase-stage has never been observed by other authors. A comparison of the number of cell-divisions in untreated and heat-treated wings, however, shows that the number of mitoses is smaller

in treated wings up to 24 hours after exposure than in untreated wings. This fact makes it more plausible to regard the effect of heat as an arrest of new cell-divisions. The cells which were already dividing finish their mitosis at a slower rate and few, if any, new cells enter the process of cell-division. Therefore fewer metaphases and many more anaphases are found. 36 hours after exposure to heat the number of cell-divisions and the metaphase-anaphase ratio are normal again.

This explanation agrees with Wassermann's observation on plant material (*Allium cepa*) and the mitosis-free period after X-radiation described by Marquardt (1937). The factor responsible for the disturbance may be dehydration due to the heat-treatment.

### Summary

Wings of pupae of *Ephestia kühniella*, which were exposed to heat at the beginning of the mitosis-period, show a decrease in number of cell divisions and a large increase of anaphases 12-24 hours after exposure. This suggests that few, if any, cells enter mitosis after heat-treatment, while the cells already dividing during the time of exposure finish their process of division at a slower rate. 36 hours after exposure to heat the normal division-process sets in again.

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## Über Vakuolenkontraktion und Anthozyanophoren bei *Pulmonaria*

Von  
Ernst Küster

Eingegangen am 29. Mai 1939

MOLISCH (1905) hat mit zahlreichen Beispielen dargetan, wie oft und wie mannigfaltig in lebenden Pflanzenzellen das Anthozyan rein oder mit anderen Verbindungen gemischt ausfallen kann. In den Kronen von *Pulmonaria* treten umfangreiche rote Pigmentkörper auf, die WEBER (1934, 1936) also Anthozyanophoren bezeichnet hat. Wir wollen im folgenden versuchen, diesen eigenartigen Anthozyanhäufungen, ihrer Entstehung und ihrem Formwechsel nachzugehen.

Die von WEBER (1934, 1936) entdeckten und beschriebenen Pigmentkörper der *P. rubra* finden sich nur in der Krone und auch in dieser nur in eng begrenzten Arealen: an der Außenseite der Krone findet sich ein Ring von Zellen, die mit Pigmentballen gefüllt sind; er liegt zwischen Kronröhre und Kronzipfel.

Die Zellen, welche mit Pigmentkörpern ausgestattet sind, können der Epidermis oder dem Grundgewebe angehören.

Selbst sehr jugendliche Blüten zeigen bereits den Pigmentkörperring. Seine Breite scheint während der Anthese zunehmen zu können; bei sehr jugendlichen Blüten fand ich ihn etwa 3 Zellen breit, bei älteren doppelt und dreimal so breit, sogar bis 20 Zellen breit. Begreiflicherweise ging es nicht an, ein und dieselbe Blüte in mehreren Phasen zu untersuchen, sodaß ich mich über den Breitenzuwachs der Pigmentkörperringe nur mit der soeben vorgetragenen Vermutung äußern kann.

Die Pigmentkörper sind niemals kugelig, sondern stets abgerundet-kantig geformt. Sie wiederholen mit ihren Umrissen die Form ihrer Zellen—freilich ohne Einzelheiten, sodaß die Undulation des Umrisses, die die Zelle kennzeichnet, an den Pigmentkörpern garnicht oder nur in bescheidener Andeutung erkennbar ist.

In ihrer Länge bleiben die Pigmentkörper erheblich hinter der der Zellen zurück; ihre Breite entspricht ungefähr der der Zellen. Die Messungen der Pigmentkörper und der Zellen wurde in der Weise durchgeführt, daß ich bei Feststellung der Breite von einem Wellental zum anderen Tal gegenüberliegender Längswände maß, sodaß die nachfolgend genannten Breitenwerte der Zellen stets die geringsten der Zellen zum Ausdruck bringen.

Zellenbreite	Pigmentkörperbreite
35,3 $\mu$ . . . . .	29,4 $\mu$ . . . . .
35,3 „ . . . . .	35,3 „ . . . . .
41,0 „ . . . . .	41,0 „ . . . . .
47,0 „ . . . . .	41,0 „ . . . . .
47,0 „ . . . . .	47,0 „ . . . . .
47,0 „ . . . . .	47,0 „ . . . . .
52,9 „ . . . . .	47,0 „ . . . . .
52,9 „ . . . . .	52,9 „ . . . . .
64,7 „ . . . . .	58,8 „ . . . . .

Die Pigmentkörper sind anscheinend zunächst relativ groß und sinken später zusammen.

Die Farbe der Pigmentkörper entspricht der des Zellsaftes. Große Pigmentkörper unterscheiden sich hinsichtlich der Intensität der Färbung nur wenig von dem Zellsaft; ihre Wahrnehmung macht infolgedessen manchmal Schwierigkeiten. Sind die Pigmentkörper klein, so ist ihr Rot wesentlich dunkler als das des sie umgebenden Zellsaftes, und die Wahrnehmung der Körper macht keine Schwierigkeiten. Eine Wirkung des Alterns der Pigmentkörper sehe ich darin, daß sie ihre ursprüngliche Klarheit verlieren und körnig und trübe werden oder sogar ein Gerüst von feinem Gerinnsel überall entwickeln. Dieselben alternden Pigmentkörper zeigen an ihrer Oberfläche oftmals eine deutlich erkennbare Membran.

Namentlich durch Trauma gelingt es, bei *P. rubra* wie bei vielen anderen Gewächsen (KÜSTER 1938) den Farbenton von rot nach violett und schließlich zu blau umschlagen zu lassen; die Farbänderung betrifft ebenso sehr die Pigmentkörper wie den sie umgebenden Zellsaft.

Durch Trauma läßt sich an den roten Zellen der *Pulmonaria* eine wohlgekennzeichnete Vakuolenkontraktion hervorrufen; es wird bei ihr der blauverfärbte Zellsaft stark eingeeengt, das Plasma quillt zu einer umfangreichen vakuolenreichen Masse heran. Sehr oft geht dieser Prozeß so weit, daß die Vakuole nur noch als dünner schwarzblauer Schlauch erscheint, die mit ihrer Form die der Zelle in allen Einzelheiten wiederholt, sodaß mit der Vakuole ein zierlich verkleinertes Abbild der ganzen Zelle vorliegt, dessen Form von der der „Pigmentkörper“ oder Anthozyanophoren sich deutlich unterscheidet. Auch an blauen Zellen, welche „Pigmentkörper“ enthalten, kann Vakuolenkontraktion eintreten. Niemals habe ich Vakuolenkontraktion der hier beschriebenen Art in unverfärbten roten Zellen gefunden, sondern nur in violetten und blauen.

Auf verschiedene Weise wurde versucht, die Anthozyanophoren zur Beseitigung zu bringen, ohne das Leben der Zelle zu schädigen. Es gelang durch Behandlung der Zellen mit alkalischen Mitteln: Sodalösung, Ammoniakdämpfen, 1%iges Koffein. Der Inhalt der

roten Zellen bläut sich dabei, bleibt aber noch am Leben, wie Versuche mit plasmolysierenden Mitteln zeigen; nur mit Koffeinelösung gelang es, rote Zellen unverfärbt zu lassen und die Pigmentkörper in ihnen zum Verschwinden zu bringen.

Bei Behandlung mit 24% Alkohol sieht man die Pigmentkörper langsam schwinden; garnicht selten wird an einer Zellenflanke der Umriß des Pigmentkörpers unsichtbar, während er auf der gegenüberliegenden noch geraume Zeit erhalten bleibt.

Ich wäre auf die an den Anthozyanvakuolen von *Pulmonaria* sichtbaren Strukturen nicht so ausführlich eingegangen, wenn nicht WEBER bedeutungsvolle Schlüsse aus seinen Befunden gezogen hätte, die ich in den Frühjahrsmonaten 1937, 1938 und 1939 nachzuprüfen Gelegenheit genommen habe. Nicht allen Folgerungen und Vermutungen, die WEBER vorgetragen hat, glaube ich mich anschließen zu dürfen.

Die roten Pigmentkörper der *Pulmonaria* nennt WEBER Anthozyanophoren—eine Bezeichnung, die insofern vielleicht nicht besonders glücklich ist, als das Anthozyan nicht nur an sie gebunden ist, sondern auch in dem sie umspülenden Zellsaft erscheint, wie WEBER bereits beschrieben hat.

WEBER erörtert, ob vielleicht die „Anthozyanophoren“ durch eine „primäre (physiologische) Vakuolenkontraktion“ zustande kommen. Zu dieser Vermutung mag vielleicht die Betrachtung der Form der Anthozyanophoren geführt haben, die die der Zellen in ähnlicher, allerdings unvollkommenerer Weise wiederholt als die unzweifelhaften „pathologischen“ Vakuolenkontraktion, die nach Trauma besonders zahlreich erscheinen. Fassen wir mit WEBER die Anthozyanophoren als Produkt einer Vakuolenkontraktion auf, so müßten wir annehmen, daß die Vakuolen auf das Volumen zurückgehen, das die von ihnen sich herleitenden Anthozyanophoren aufweisen, und die Vakuolenhüllen auf den Grenzfläche der letzteren und des ihn umgebenden roten Zellsaftes zu suchen seien, auf der nicht selten später eine derbe Niederschlagsmembran deutlich wahrnehmbar wird. Eine Vakuolenkontraktion solcher Art liegt indessen nach WEBER's Auffassung wohl nicht vor, vielmehr ein Vorgang, den STRUGGER (1935, 107) „als eine durch Synaerese bewirkte Sol- und Gelteibildung im Zellsaft“ anzusprechen vorzieht.

Derselbe Forscher ist der Meinung, daß die für die Korollen der Boraginazeen wohl bekannten Vakuolenkontraktionen ohne Beteiligung des Protoplasmas zustande kommen. Dieser Meinung darf ich mich nicht anschließen; vielmehr sehe ich bei der Vakuolenkontraktion unseres Objektes das Protoplasma in derselben Masse und in derselben Weise in Aktion treten wie bei der des *Allium*typus;



Schwierigkeiten für den Nachweis seiner Wandlungen macht freilich die starke Vakuolisierung, die seiner Quellung folgt; wenn wir ein dunkelblaues zierlich geformtes Gebilde in den Boraginazeenzellen finden, so liegen diese nicht in farblosem Zellsaft, sondern in Protoplasma, allerdings in einem nicht nur sehr wasserreichen, sondern auch stark vakuolisierten. Es ist nicht immer leicht, sich von dem Vorhandensein einer dünnen Schicht von Protoplasma zu überzeugen, das dem stark kontrahierten Vakuolenkörper anliegt und ihn umgibt, und in dem zuweilen kleine Plastiden erkennbar sind, und das manchmal zarte Fäden mit dem wandständigen Plasmabelag verbinden (KÜSTER 1938, 33). Ein Objekt, an dem Vakuolenkontraktion derselben Art sich vollzieht, an dem ganz ähnlich starke Vakuolisierungen des Protoplasmas sich abspielen, der Nachweis der Plasmakonfiguration des zwischen Vakuolenkörper und Plasmawandbelag liegenden Anteiles des Zelleninhalts aber leichter gelingt, habe ich mit den Zellen des Perigons von *Hyacinthus* (1938a) beschrieben.

Vielleicht führt uns ein Vergleich der bei *P. rubra* beobachteten Strukturen mit dem, was uns andere *P.* Arten zeigen, einen Schritt weiter.

Bei *Pulmonaria officinalis* liegen ähnliche Verhältnisse vor wie bei *P. rubra*—WEBER hat bereits auf diese Übereinstimmungen hingewiesen.

Dort, wo der gefärbte Teil der Krone von *P. officinalis* an die nahezu farblose Kronenröhre grenzt, findet sich eine ringförmige Zone, in der die Zellen der nach außen gewandten Epidermen rote Inthaltkörper zeigen, die nur unvollkommen die Form der Zelle wiederholen. Oftmals sind sie viereckig gestutzt und zeigen kaum eine formale Abhängigkeit von den undulierten Umrissen der Epidermiszellen. Von den Anthozyanophoren der *P. rubra* unterscheiden sie sich dadurch, daß nicht anthozyanfarbener Zellsaft, sondern ein farbloses Medium sie umgibt, und daß ihre Kontraktion in der Breitenausdehnung der Zelle stärker ist, als sie bei *P. rubra* zu sein pflegt. Neben diesen oft sehr zahlreichen Zellen finden sich andere, bei welchen ein ebenso gestalteter Körper in zellrotem Zellsaft liegt, und weitere Zellen, bei welchen ein blauer Pigmentkörper in hellblauem Zellsaft liegt, und schließlich stark kontrahierte dunkelblaue Körper in farbloser Umgebung; die letzteren werden wir unbedenklich als Vakuolenkontraktionen ansprechen, die anderen mit den oben besprochenen Gelmassen STRUGGER's gleichzustellen geneigt sein, falls nicht die erste Gruppe von Erscheinungen uns daran hindert. Ist die farblose Umgebung des roten Körpers wasserreiches vakuoliges Protoplasma? Oder haben wir mit ihr den Solanteil des ent-

mischten Zellsaftes vor uns, der hier—im Gegensatz zu *P. rubra*—keinen Rest des Farbstoffgehalts in sich zu bewahren vermag: im ersten Fall würden wir auf Vakuolenkontraktion, im anderen auf Gelentmischung zu schließen haben. Mit der Annahme einer Vakuolenkontraktion schlecht vereinbar scheint die rote Farbe zu sein; der alkalische Farbton des Anthozyans ist als Begleiterscheinung, vielleicht als Voraussetzung der Vakuolenkontraktion für viele Objekte bekannt (WEBER 1936; KÜSTER 1938; DRAWERT 1938); doch sind auch Kontraktionen an roten Anthozyanvakuolen schon beobachtet worden (KEIL 1930); sie fehlen auch bei *P. officinalis* nicht. Der Kern, der in dem farblos erfüllten Raum liegt, zeigt dasselbe Bild, das wir von vielen Vakuolenkontraktionen her kennen.

Ich kann mich nicht dazu entschließen, die in farblosem Medium liegenden Körper für Produkte einer Vakuolenkontraktion, die in roter Flüssigkeit liegenden für die einer Zellsaftentmischung zu halten; die Bilder, die die Zellen in beiden Fällen gewähren, stimmen in allen anderen Punkten vollkommen miteinander überein, so daß ich sie für Produkte gleicher oder ähnlicher, nicht grundsätzlich verschiedener Vorgänge halten möchte. Für diese und jene eine einheitliche Erklärung gelingt m.E. auf folgendem Wege.

Ich nehme an, daß alle hier beschriebenen Bilder auf Vakuolenkontraktion zurückgehen; die Vakuolen verkleinern sich mehr oder weniger, das sie umgebende Protoplasma gewinnt an Ausdehnung und vakuolisiert sich stark. Die neugebildeten Vakuolen erfüllt—wie in vielen anderen bereits erforschten Fällen „pathologischer“ Vakuolenbildung—ein farbloser Zellsaft. Dieser bleibt aber nur farblos, solange die kontrahierte Vakuole umspannende plasmatische Membran die Anthozyanlösung zurückhält; in späteren Stadien der Entwicklung diffundiert bei *P. officinalis* der Farbstoff in den Zellsaft der neugebildeten Vakuolen und färbt sie rot. Der kontrahierte Vakuolenkörper zeigt bei *P. officinalis* in dieser Phase Anzeichen degenerativer Veränderungen, er wird körnig und besteht schließlich oft aus einem gerüstähnlichen farbigen Körper, der keine geschlossene Oberfläche mehr erkennen läßt.

Aus den Bildern, die das Grundgewebe der Korollen von *P. officinalis* zeigt, schließe ich, daß auch Veränderungen anderer Art an dem kontrahierten Vakuolenkörper sich abspielen können: ich fand in rotvioletter Zellsaft blaue Vakuolenkörper, die zu kleinen, faltigen, eingedellten Säckchen zusammengeschrumpft waren.

Die Färbung der im gequollenen Protoplasma liegenden Vakuolen erfolgt bei *P. rubra* offenbar früher als bei *P. officinalis*. Ist sie eingetreten, so kann die Farbstoffabgabe an die äußeren Vakuolen so reichlich erfolgen, daß der Farbunterschied zwischen dem „Antho-

zyanophoren“ und dem diesen umgebenden Medium gering wird.

Auch an Zellen, die diese Veränderungen hinter sich haben, läßt sich erneut Vakuolenkontraktion hervorrufen—durch Trauma. Bringt man den Korollenblättern in der Nähe der uns beschäftigten Zellen kleine Stichwunden bei, so sieht man hie und da den gesamten anthozyanhaltigen Raum der Zelle sich verkleinern und eine peripherische äußere Schicht an Dicke gewinnen. Daß es sich hierbei um Vakuolenkontraktion handelt, ist durch vorsichtige Plasmolyse darzutun; der Wandbelag des Protoplasmas wird nach Kontraktion sichtbar.

Es entspricht den für *Allium cepa* (KÜSTER 1927) bekannten Vorgängen, wenn die Ausdehnung des farblosen Anteils sich nur an dem wundfernen Ende der langgestreckten Zelle abspielt—und das dem Trauma zugewandte zunächst unverändert bleibt.

Einige Male habe ich Zellen wahrgenommen, in welchen der peripherische von Protoplasma, vermutlich von wiederum früh vakuolisiertem, erfüllt war, schwach farbig zu werden schienen—wie ich annehme, weil die den anthozyanhaltigen Zellsaftum umhüllende Schicht zerstört oder permeabel wurde. Leider war die Farbabgabe eines violett oder blau verfärbten Zellsaftes nicht mit der Zuverlässigkeit zu konstatieren, daß ich mich über die beobachteten Fälle anders als mit Vermutungen äußern könnte.

Ungewöhnliche Formen von Vakuolenkontraktion werden an Zellen sichtbar, die 24 Stunden in  $n/2$  Harnstoff gelegen haben, hiernach mit reinem Wasser behandelt worden sind. Es erfolgt sofort starke Vakuolenkontraktion,—und zwar derart, daß an irgendwelchen Stellen die Vakuole nicht von ihrem normalen Ort abrückt, sondern im Umrisse der Zelle liegen bleibt; so entstehen ähnliche Bilder wie ich sie (1938a) für *Hyacinthus* beschrieben habe.

### Zusammenfassung

1) Anthozyanophoren (im Sinne WEBERS) werden für *Pulmonaria rubra* und *P. officinalis* beschrieben.

2) Die Form der Anthozyanophoren wiederholt im wesentlichen die der Zellen.

3) Die Bildung der Anthozyanophoren wird auf Vakuolenkontraktion, nicht auf Gel- und Soltrennung des Zellsaftes zurückgeführt.

4) Vakuolenkontraktion spielt sich stets unter Beteiligung des Protoplasmas ab, indem dieses stark quillt oder nach Vakuolisierung schwillt. Vorgänge, die ohne Beteiligung des Protoplasmas an der Vakuole sich abspielen, als Vakuolenkontraktion zu bezeichnen, scheint nicht zweckmäßig.

5) Auch an roten (sauren) Zellen kann bei *Pulmonaria* Vakuolenkontraktion eintreten.

6) Nach Vakuolisierung des Protoplasmas und Verkleinerung des anthozyanreichen Zellsaftraumes kann Farbstoff von diesem an die sekundär gebildeten farblosen Vakuolen abgegeben werden.

7) Vakuolenkontraktion scheint für die Korollen der *Pulmonaria* oder für bestimmte Anteile derselben ein physiologischer Vorgang zu sein—auch sehr junge Blüten zeigen ihn; an bestimmten Anteilen der Krone enthält jede Zelle einen durch Vakuolenkontraktion entstandenen Anthozyankörper.

8) Neben der physiologischen Vakuolenkontraktion kann sich gleichzeitig in denselben Korollengewebe eine pathologische abspielen—z.B. nach Trauma. Bei dieser sind die Vakuolen meist dunkelviolett oder dunkelblau gefärbt. Die pathologische Kontraktion führt zu erheblich schlankeren, stärker undulierten Formen als die „physiologische“; eine Abgabe von Farbstoff an die farblose Umgebung tritt zu Lebzeiten der Zelle nach pathologischer Kontraktion nicht ein.

9) Zellen, deren Vakuolen physiologische Kontraktion erfahren haben, können durch Trauma zu erneuter pathologischer Vakuolenkontraktion gebracht werden; hierbei erfolgt zuerst Farbumschlag nach violett und blau.

10) Die durch Trauma bedingte pathologische Kontraktion erfolgt einseitig; d.h. derart, daß an der wundnahen Seite der Rand der Vakuole seine normale Lage behält, und nur an der wundenfernen der Abstand zwischen jener und der Membran sich vergrößert.

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## Chromosome Studies in Cyperaceae, IV Chromosome number of *Carex* species<sup>1)</sup>

By

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(With 34 text-figures)

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The genus *Carex* has attracted particular notice in its remarkable aneuploidy of chromosome numbers which have been reported by Heilborn (1922, 1924, 1925, 1932), Wulff (1933) and recently by the writer (1937). There have been found 28 different chromosome numbers, ranging 9 to 56, which do not show any multiple relation, namely, 9, 15, 16, 18, 19, every number of 23–43, 54 and 56 (in haploid number). From the view point of the chromosome evolution or species formation it may be noteworthy to investigate still more number of species, especially more precise chromosomal relationships among species by crossing them artificially with one another. The writer has made crosses of some 13 species in various combinations this Spring (1938) and obtained a number of species hybrids in 19 combinations which will be reported later in a paper of this series. In the present paper, a preliminary report on the chromosome numbers of 32 species and 3 varieties will be made.

### Materials and Methods

Materials were collected in various districts: in the vicinity of Tokyo; Hakone, and Mt. Ōyama in Sagami Province; Mt. Kiyosumi-yama in Tiba Prefecture; Nikkō in Totigi Prefecture; Mt. Idugatake in Musasi Province; Mt. Yatugatake in Nagano Prefecture; Adiro in Siduoka Prefecture; and Mt. Taisetuzan in Hokkaidō.

The root-tips were fixed with Navashin's chrom-acetic solution for 24 hours, and sectioned by usual paraffin method. For staining the gentianviolet iodine method according to Newton's schedule was applied. The inflorescences were fixed with Carnoy's fluid, and they were preserved in 75% alcohol, after being washed in 95% alcohol, until necessary. Meiosis was observed by the aceto-carmin smear method.

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## Observations

Chromosome numbers counted were summarized in table 1, in which all species and varieties were arranged after Ohwi's system (1936). A few species will be briefly described here.

Table 1

Genus <i>Carex</i> Linn.	n	2n (Fig.)
Subgenus <i>Vignea</i> Nees		
Sect. Multiflorae Kunth		
<i>Carex stipata</i> Mühlenb. (Jn. <sup>1)</sup> <i>Ōkawazusuge</i> (Mt. Yatugatake)	48	(11)
Sect. Gibbae Kükenth.		
<i>C. gibba</i> Wahlenb. { (Mt. Ōyama, Hakone, Adiro)	34	(7a)
(Jn. <i>Masukusa</i> ) { (Vicinity of Tokyo)	36	(7b)
Sect. Elongatae Kunth		
<i>C. Rochebruni</i> Franch. et Savat. (Jn. <i>Yabusuge</i> ) (Vicinity of Tokyo)	64	(23)
Subgenus <i>Eucarex</i> Coss. et Germ.		
Sect. Acutae Fries		
<i>C. incisa</i> Boott (Jn. <i>Kawarasuge</i> ) (Hakone, Vicinity of Tokyo)	68	(25)
<i>C. Thunbergii</i> Steud. (Jn. <i>Azesuge</i> ) (Nikkō, Vicinity of Tokyo)	78	(33)
<i>C. Maximowiczii</i> Miq. (Jn. <i>Gōso</i> ) (Hakone, Mt. Yatugatake)	74	(28)
<i>C. kiotensis</i> Franch. et Savat. (Jn. <i>Tekirisuge</i> ) (Mt. Ōyama, Hakone, Nikkō)	74	(27)
Sect. Atratae Kunth		
<i>C. curvicolis</i> Franch. et Savat. (Jn. <i>Narukosuge</i> ) (Mt. Idugatake)	28	— (6)
<i>C. Tolmiei</i> Boott var. <i>denticulata</i> Ohwi (Jn. <i>Miyamakurobosuge</i> ) (Mt. Yatugatake)	62	(17)
Sect. Ferginae Tuckerm.		
<i>C. stenantha</i> Franch. et Savat. (Jn. <i>Iwasuge</i> ) (Mt. Yatugatake)	68	(26)
Sect. Decoraе Kükenth.		
<i>C. Reini</i> Franch. et Savat. (Jn. <i>Kokansuge</i> ) (Mt. Ōyama, Hakone, Mt. Idugatake)	26	(5)
Sect. Praecoces Christ.		
Subsect. Mitratae (Kükenth.) Ohwi		
<i>C. nervata</i> Franch. et Savat. (Jn. <i>Sibasuge</i> ) (Adiro, Hakone, Mt. Ōyama)	76	(29)
<i>C. breviculmis</i> R. Br. (Jn. <i>Aosuge</i> ) (Adiro, Hakone, Mt. Kiyosumiyama, Mt. Yatugatake)	34	64 (24)
<i>C. clivorum</i> Ohwi (Jn. <i>Yamaōitosuge</i> ) (Mt. Yatugatake)	76	(30)
<i>C. alterniflora</i> Franch. (Jn. <i>Ōitosuge</i> ) (Adiro, Mt. Kiyosumiyama, Mt. Idugatake)	76	(31)
<i>C. Duvaliana</i> Franch. et Savat. (Jn. <i>Kesuge</i> ) (Mt. Ōyama, Adiro, Mt. Kiyosumiyama)	76	(32)

1) Jn. = Japanese name.

	n	2n	(Fig.)
<i>C. conica</i> Boott { (Mt. Ōyama, Mt. Idugatake) {	34	8a	
(Jn. <i>Himekansuge</i> ) {	35	8b	
(Mt. Kiyosumiyama, Mt. Idugatake)	38	8c	
<i>C. Morrowii</i> Boott (Jn. <i>Kansuge</i> ) (Hakone, Mt. Kiyosumiyama, Mt. Idugatake)	38	9	
Sect. Rhomboidales Kükenth.			
<i>C. Bootiana</i> Hook. et Arn. (Jn. <i>Isosuge</i> ) (The Koisikawa Botanic Garden in Tokyo)	31+f	62	(19)
<i>C. insanae</i> Koidzumi var. <i>papillaticulmis</i> Ohwi (Jn. <i>Aobasuge</i> ) (Mt. Kiyosumiyama)	50	13	
Sect. Digitatae Fries			
<i>C. lasiolepis</i> Franch. (Jn. <i>Adumasuge</i> ) (Mt. Kiyosumiyama, Mt. Idugatake)	8	16	(2a b)
Sect. Grallatoriae Kükenth.			
<i>C. grallatoria</i> Maxim. var. <i>heteroclita</i> Kükenth. et Matsum. (Jn. <i>Sanagisuge</i> ) (Mt. Idugatake)	9	18	(3)
Sect. Montanae Fries			
<i>C. oxyandra</i> Kudo (Jn. <i>Himesuge</i> ) (Nikkō, Mt. Idugatake)	9	18	(4)
Sect. Paniceae Tuckerm.			
<i>C. macroglossa</i> Franch. et Savat. (Jn. <i>Mugisuge</i> ) (Vicinity of Tokyo)	50	12	
Sect. Sideroatictae Franch.			
<i>C. sideroaticta</i> Hance (Mt. Idugatake)	6	12	(1a)
(Jn. <i>Taganesō</i> ) { (Nikkō, Mt. Yatugatake)	12	24	(1b)
Sect. Ischnostachyae Ohwi			
<i>C. ischnostachya</i> Steud. (Jn. <i>Zyudusuge</i> ) (Mt. Kiyosumiyama)	62	16	
Sect. Rhizopodae Ohwi			
<i>C. rhizopoda</i> Maxim. (Jn. <i>Sirakosuge</i> ) (Vicinity of Tokyo)	64	22	
Sect. Molliculae Ohwi			
<i>C. japonica</i> Thunb. (Jn. <i>Higokusa</i> ) (Vicinity of Tokyo)	62	15	
<i>C. planiculmis</i> Komar. (Jn. <i>Hikagesirasuge</i> ) (Mt. Taise-tuzan)	62	18	
Sect. Paniceae Ohwi			
<i>C. satsumensis</i> Franch. et Savat. (Jn. <i>Aburasiba</i> ) (Mt. Ōyama, Nikkō)	19	38	(10)
Sect. Graciles Tuckerm.			
<i>C. brunnea</i> Thunb. var. <i>Nakiri</i> Ohwi (Jn. <i>Nakirisuge</i> ) (Mt. Ōyama, Hakone, Adiro, Mt. Idugatake)	62	20	
Sect. Confertiflorae Franch.			
<i>C. Idzuroei</i> Franch. et Savat. (Jn. <i>Umasuge</i> ) (Vicinity of Tokyo)	58	14	
Sect. Dispalatae Ohwi			
<i>C. dispalata</i> Boott (Jn. <i>Kasasuge</i> ) (Vicinity of Tokyo)	78	34	
Sect. Paludosae Fries			
<i>C. pumila</i> Thunb. (Jn. <i>Kōbōsiba</i> ) (The Koisikawa Botanic Garden in Tokyo)	41	82	
Sect. Physicarpae Drejer			
<i>C. Dickinsii</i> Franch. et Savat. (Jn. <i>Onisuge</i> ) (Mt. Yatugatake)	64	21	

*Carex siderosticta* Hance (Figs. 1a, b) ( $2n = 12$ ;  $n = 12$ ,  $2n = 24$ ). The plants collected at Mt. Idugatake were diploid having 12 chromosomes in their root-tip cells, while those collected at Nikkō were tetraploid having 24 chromosomes in their root-tip cells. The tetraploid form contains double chromosome sets of the diploid form which has 8 long (L) and 4 short (S) chromosomes. Two long chromosomes out of the 8 L possess satellites. Granting the medium size of L ( $3.18 \mu$ ) as 100, the mean relative lengths of 8 L and 4 S equal to  $94.95 \pm 2.02$  ( $\sigma = \pm 11.4$ ) and  $58.45 \pm 1.63$  ( $\sigma = \pm 6.51$ ), respectively. From the point of chromosome behaviour in meiosis, this tetraploid form is considered as an autotetraploid. As regards this autotetraploid plant a more precise report will be made elsewhere. The haploid number 6 ( $1 L^t + 3 L + 2 S$ ) found in this species is the smallest one hitherto known in this genus, and it is a remarkable fact that polyploid forms have been found in this species.

*C. lasiolepis* Franch. (Figs. 2a, b). This species has 16 chromosomes ( $4 L + 2 L^t + 10 S$ ) in somatic cells. Granting the medium size of L ( $2.58 \mu$ ) as 100, the mean relative lengths of 6 L and 10 S were  $98.11 \pm 0.77$  ( $\sigma = \pm 7.33$ ) and  $51.32 \pm 9.5$  ( $\sigma = \pm 6.13$ ), respectively. Fig. 2b shows a metaphase plate of the primary pollen nuclear division, where 3 large and 5 small chromosomes can be seen.

*C. grallatoria* Maxim. var. *heterolita* Kükenth. ex Matsum. (Figs. 3a, b) has 18 chromosomes in the root-tip cells, and 9 chromosomes in the primary pollen nuclear division. In the I-metaphase of the maturation division 9 to 16 chromosomes were observed (cf. table 2). Rather feeble chromosomal affinity may be due to the instability of this species.

Table 2. Chromosome configurations in the I-metaphase of *C. grallatoria* var. *heterolita*

No. of chromosomal elements	9	10	11	12	13	14	15	16	Total
No. of PMCs observed	1	4	10	11	41	40	29	13	149

*C. oxyandra* Kudo (Fig. 4) ( $2 L^t + 4 L + 8 M + 4 S$ ) was collected at Nikkō and Mt. Idugatake. Nine bivalents in meiosis and 18 chromosomes in the root-tip cells have been observed. Granting the medium size of L ( $3.27 \mu$ ) as 100, length proportions among L, M and S are  $99.833 \pm 0.947$  ( $\sigma = \pm 6.63$ ),  $73.5 \pm 0.207$  ( $\sigma = \pm 7.665$ ), and  $40.3 \pm 1.07$  ( $\sigma = \pm 6.076$ ), respectively.

*C. Reinii* Franch. et Savat. (Fig. 5) was collected at Mt. Ōyama, Hakone and Mt. Idugatake. In the root-tip cells 26 chromosomes ( $4 L + 14 M + 8 S$ ) were counted. Length relations among L, M



and S are  $98.96 \pm 2.31$  ( $\sigma = \pm 8.0$ ),  $62.35 \pm 1.21$  ( $\sigma = + 7.83$ ), and  $30.1 \pm 1.21$  ( $\sigma = \pm 5.96$ ), respectively (where  $100 = 2.42 \mu$ ).

*C. gibba* Wahlenb. (Figs. 7a, b) was collected at Mt. Ōyama, Adiro, Hakone and in the vicinity of Tokyo. One plant collected in the vicinity of Tokyo had 34 chromosomes (Fig. 7a) in the root-tip cells, while all the rest had 36 chromosomes (Fig. 7b). Observations in meiosis of both types will be indicated later in another paper.

*C. conica* Boott (Figs. 8a, b, c). In this species also aneuploid chromosome numbers have been observed. In the root-tip cells of one plant collected at Mt. Kiyosumiyama 38 chromosomes were counted (Fig. 8c). In plants collected at Mt. Idugatake, two more different chromosome number i.e. 35 and 34 were found. A preliminary survey of meiosis of a plant with 38 somatic chromosomes, has shown some irregularities which resulted in various pollen grains. In the metaphase of the primary pollen nuclear division, 15 to 20 chromosomes were counted (cf. table 3) and this fact agreed with the result of the test of germination of the pollen grains, where only 14.3% of the grains normally germinated. Further investigations in a larger scale will be reported in future.

Table 3. Chromosome numbers in the pollen grains of *C. conica*

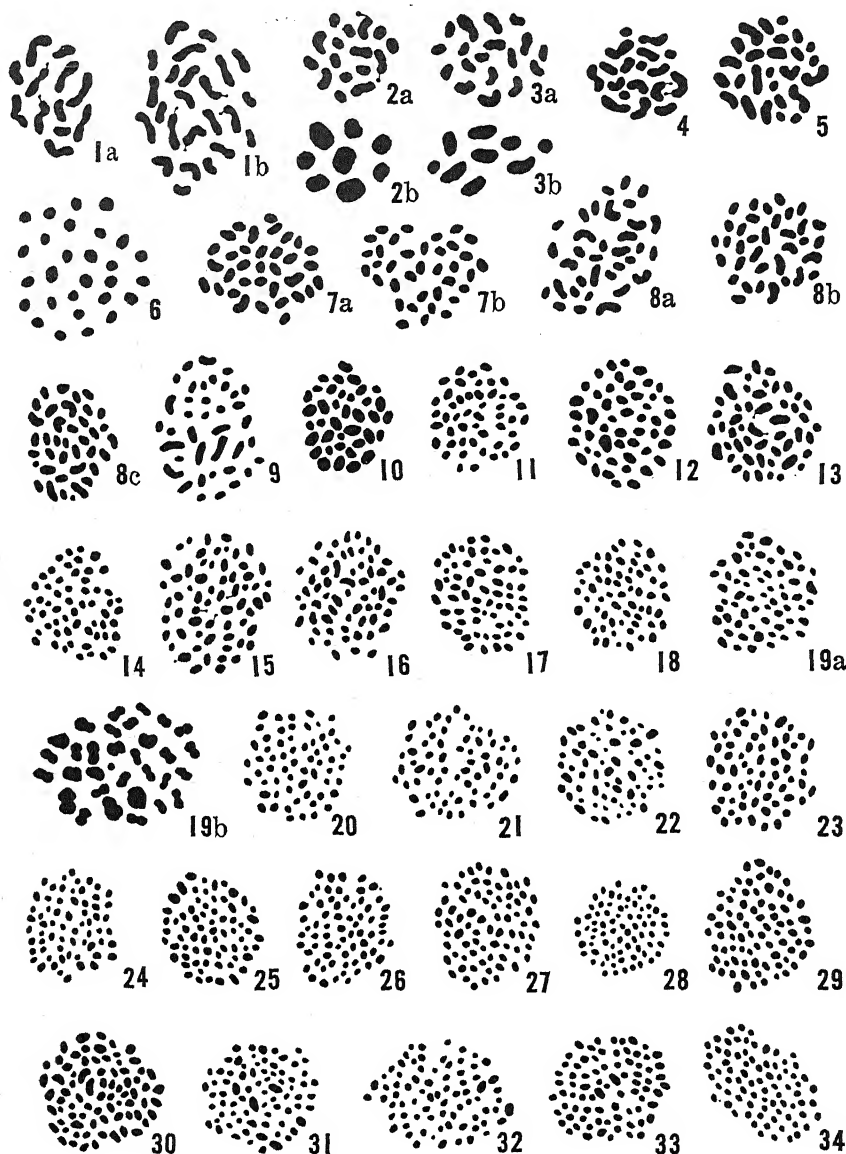
No. of chromosomal elements	15	16	17	18	19	20	Total
Frequency	1	3	11	16	16	4	51

*C. breviculmis* R. Br. (Fig. 24). This species has a wide distribution in Japan and shows a considerable variation in its phenotype. Some of them have shown 34 chromosomes in the I-metaphase of the maturation division, while the other had 64 chromosomes in the root-tip cells.

*C. Boottiana* Hook. et Arn. (Figs. 19a, b). Materials were taken from the plants cultivated in the Koisikawa Botanic Garden of the Tokyo Imperial University. In the root-tip cells 62 chromosomes were counted, while in the I-metaphase of the maturation division one chromosomal fragment has been observed usually.

*C. pumila* Thunb. Chromosome number of this species was reported as  $n = 41$  in the writer's earlier paper (1937), and it is now verified in the root-tip cells where 82 chromosomes have been clearly counted.

*C. satsumensis* Franch. et Savat. (Fig. 10) was collected at Mt. Ōyama and Nikkō. In the root-tip cells 38 chromosomes were counted. Hence the haploid number 19 reported in the previous paper (1937) has been confirmed.



Figs. 1-34. 1a, b. *C. siderosticta*; 1a, diploid form  $2n=12$  and 1b, tetraploid  $2n=24$ . 2a, b. *C. lasiolepis*. a, metaphase of the somatic mitosis  $2n=16$ . b, metaphase of the pollen nuclear division  $n=8$ . 3a, b. *C. grallatoria*. a, metaphase of the root-tip cells  $2n=18$ . b, pollen nuclear division,  $n=9$ . 4, *C. oxyandra*  $n=18$ . 5. *C. Reinii*  $2n=26$ . 6, *C. curvifolia*, pollen nuclear division,  $n=28$ . 7a, b. *C. gibba*. a,  $2n=34$ . b,  $2n=36$ . 8a, b, c. *C. conica*. a,  $2n=34$ . b,  $2n=35$ . c,  $2n=38$ . 9. *C. Morrowii*  $2n=38$ . 10. *C. satsumensis*  $2n=38$ . 11. *C. stipata*  $2n=48$ . 12. *C. maroglossa*  $2n=50$ . 13. *C. insanae* var. *papillaticulmis*  $2n=50$ . 14. *C. Idzuroei*  $2n=58$ . 15. *C. japonica*  $2n=62$ . 16. *C. ischnostachya*  $2n=62$ . 17. *C. Tolmiei* var. *denticulata*  $2n=62$ . 18. *C. planiculmis*  $2n=62$ . 19a, b. *C. Boottiana*. a, root-tip cells  $2n=62$ . b, meiosis with  $31_{II}+1f$ . 20. *C. brunnea* var. *Nakiri*  $2n=62$ . 21.

### Considerations

In the present work the haploid number of 6, 8, 12, 13 and 17 are newly added to the series of the chromosome numbers of the genus *Carex*. It is noteworthy that aneuploidy is also present in the section or subsection. Hitherto no polyploid species has been found in this genus, with one exception of *Carex glauca* Murr. (Heilborn 1924, 1932) which has been considered as a secondary balanced autotetraploid species derived from a  $2n=16$  ancestral form. However, two more examples with polyploid forms have been found by the present work as described above, i.e. *Carex siderosticta* and the subsection *Mitratae*. In the latter two chromosome numbers 76 and 38 which abide by the law of multiples have been discovered. Therefore it may be expected that the ordinal polyploidy is found more often in the genus *Carex* than considered until now and that natural hybrids exist in a certain extent in this genus.

The earlier investigations of the chromosome number have been mainly made only in the maturation divisions, which sometimes made erroneous in counting chromosome numbers, because there seems produced a rather large number of natural hybrids in *Carex*. In fact more than two chromosome numbers have been reported as in the case of *C. gibba*, *C. conica* and *C. breviculmis*. The fact that species hybrids have actually been obtained by the writer in the following 19 combinations (unpublished) may strongly suggest the possibility of the occurrence of natural hybrids: *C. lasiolepis*  $\times$  *C. Morrowii*, *C. lasiolepis*  $\times$  *C. Reinii*, *C. Morrowii*  $\times$  *C. conica*, *C. conica*  $\times$  *C. Morrowii*, *C. conica*  $\times$  *C. Reinii*, *C. alterniflora*  $\times$  *C. Duvaliana*, *C. Duvaliana*  $\times$  *C. Morrowii*, *C. Duvaliana*  $\times$  *C. conica*, *C. Boottiana*  $\times$  *C. Morrowii*, *C. oxyandra*  $\times$  *C. Morrowii*, *C. curvicolis*  $\times$  *C. lasiolepis*, *C. curvicolis*  $\times$  *C. siderosticta* (2b), *C. siderosticta* (4b)  $\times$  *C. conica*, *C. siderosticta* (4b)  $\times$  *C. breviculmis*, *C. siderosticta* (4b)  $\times$  *C. curvicolis*, *C. lanceolata*  $\times$  *C. lasiolepis*, *C. lanceolata*  $\times$  *C. Morrowii*, *C. lanceolata*  $\times$  *C. siderosticta* (2b), *C. insanae* var. *papilaticulmis*  $\times$  *C. lasiolepis*.

Consequently more precise investigations about the chromosome numbers must be made in both meiosis and somatic mitosis of many species in order to interpret correctly the phenomenon of aneuploidy of this genus.

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- C. Dickinsonii*  $2n=64$ . 22. *C. rhizopoda*  $2n=64$ . 23. *C. Rochebruni*  $2n=64$ . 24. *C. breviculmis*  $2n=64$ . 25. *C. incisa*  $2n=68$ . 26. *C. stenantha*  $2n=68$ . 27. *C. kiotsensis*  $2n=74$ . 28. *C. Maximowiczii*  $2n=74$ . 29. *C. nervata*  $2n=76$ . 30. *C. clivorum*  $2n=76$ . 31. *C. alterniflora*  $2n=76$ . 32. *C. Duvaliana*  $2n=76$ . 33. *C. Thunbergii*  $2n=78$ . 34. *C. dispalata*  $2n=78$ .

### Summary

The chromosome numbers of 32 species and 3 varieties of the genus *Carex* have been reported. Five haploid numbers, 6, 8, 12, 13 and 17 are newly added to the series of known chromosome numbers of this genus, hence we have the total of 33 different haploid chromosome numbers, namely, 6, 8, 9, 12, 13, 15, 16, 17, 18, 19, every number of 23-43, 54 and 56.

The karyotypes of *Carex siderosticta*, *C. lasiolepis*, *C. oxyandra* and *C. Reinii* have been determined as follows,  $1L^t + 3L + 2S$ ,  $3L + 5S$ ,  $1L^t + 2L + 4M + 2S$ ,  $2L + 7M + 4S$ , respectively.

In 3 species aneuploid chromosome numbers have been found, i.e. in *Carex gibba*  $2n = 34$  and  $36$ , in *C. breviculmis*  $n = 34$  and  $2n = 64$  and in *C. conica*  $2n = 34$ ,  $35$  and  $38$ . The ploid relation has also been found in a single species, *C. siderosticta*, and also in the subsection Mitratae.

From the fact that artificial species hybrids have been obtained in 19 combinations (37.3% of all crosses actually made) of 13 species, it is suggested that natural hybridization may often occur and it must have a relation to the considerable extent to the origin of aneuploidy which is too complex to interpret satisfactorily in this genus.

Here the writer wishes to record his cordial thanks to Prof. Y. Sinotô under whose direction the work has been carried out. Thanks are also due to Dr. S. Akiyama, Dr. H. Hara, Dr. F. Maekawa who kindly identified most of the plants used, and to Dr. Ohwi who had interest in the present work.

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## Cytological Studies in the Genus *Gladiolus*<sup>1)</sup>

By

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### Introduction

The genus *Gladiolus* belongs to the family Iridaceae allied to Liliaceae. It is strange, however, that the chromosomes of *Gladiolus* are extremely small in contrast to the largest known chromosomes in the Liliaceae. Probably it is this minute size of the chromosomes that has not encouraged significant cytological research in the genus. De Vilmorin and Simonet (1927) first reported the haploid number of *G. primulinus* var. hort. *La Muerthe*, as 30. Pfeiffer (1931) studied the life history of *Halley* and *Alice Tiplady*, the garden hybrids of *G. primulinus*. Schwarzenbach (1931) gave a genealogical table of a few crosses, together with a brief morphological description of the species. He ascertained the haploid number of *G. tristis* var. *colvillii* and *G. cardinalis*, as 15. The same author reports the summer *Gladioli* *G. byzantinus*, *G. gandavensis*, *G. lemoninei* and *G. nanceianus* as having  $n = 30$ . He further finds *G. primulinus* and its varieties as containing 'probably' the same number. Brittingham (1934) confirms Schwarzenbach in finding the diploid number of *G. tristis* as 30. In *Freesia refracta*, which is synonymous with *G. corymbosus*, he finds chromosomal chimaeras in the root-tip of 'Purity', a variety of the species. Since 1934, *Gladiolus* has not attracted the attention of cytologists.

It was thought proper to undertake a cytological study of a few species of the genus. The work was carried out under Professor R. Ruggles Gates, in the Botany Department, King's College, London.

From a study of four species and one variety it is suggested that the basic number of the genus is 10, not 15 as previously reported. The somatic and meiotic numbers of one species have been determined for the first time. The behaviour of the nucleolus is observed. Also the presence of prochromosomes is established and their history traced. In addition, cytomixis has been described for the first time in the genus and the several abnormalities leading to pollen sterility and clonal type of reproduction have been discussed.

1) Part IV of Thesis approved for the Degree of Doctor of Philosophy in the University of London.

## Material and Methods

Corms of four species, *G. tristis*, Salisb., *G. byzantinus* Bankin, *G. primulinus*, Baker, its variety Maiden's Blush, and *G. dracocephalus*, Hook., were variously collected from Barr and Sons, Kew, and the Chelsea Physic Gardens. They were grown in the greenhouse for root-tips and at the Regent's Park Laboratory for flower buds. Following were the fixatives used:— 1) 2BE, 2B. 2) Benda's modification of Flemming and 3) Navashin.

Benda and Navashin gave very good fixation. The time of maximum division was ascertained after several trials of whole day fixation at intervals of 3 hours. None of the species shows vigorous division at any time of the day. 10–45 P.M.—12 Midnight was found to be the most favourable time for maximum mitosis, although the rate of nuclear activity is in no way comparable to that of *Allium*. The bulbs make roots without difficulty. When the root-tips were 1–2 cm. long they were fixed continuously from 10–45 P.M.—12 Midnight, at intervals of 15 minutes. It may be stated with confidence that any time after 11 P. M. until 12 Midnight is good enough for all stages, especially metaphase plates. No pump was used at any time. Flower buds were collected from two species, *G. primulinus* and *G. dracocephalus* from Regent's Park. Fixation and after treatment were as described for *Allium* (Mensinkai 1939c) except for the Feulgen–Light Green Technique, which was after Semmens and Bhaduri (1939).

All figures in this paper are drawn at table level with the aid of a Reichert camera lucida using a 2 mm. apochromatic objective N.A. 1.4, an aplanatic condenser N.A. 1.4, and a Zeiss compensating ocular  $\times 20$  giving a total magnification of approximately  $\times 2900$ . Figs. 6–9 magnification  $\times 3400$ . Figs. 40–42 magnification  $\times 1400$ . All figures are reduced to two-thirds.

Root-tips figs. 1–38 and P.M.C. figs. 39–48 are fixed in Benda except fig. 3 which is fixed in alcohol-acetic 3:1 and stained in Feulgen and Light Green.

## Observations

Of the several species examined, *G. tristis* is the spring *Gladiolus* flowering in about 3–4 months, in February–March when planted in December. The rest are summer *Gladioli* and take three to four months to flower after planting in March–April.

*G. tristis* Salisb. has its habitat in South Africa at the Cape of Good Hope, in Paarl Mountains at 900 ft. elevation and at the foot of Van Stadens Berg below 1000 ft.

*G. primulinus* Baker. Although Baker regards it as a form of *G. quartinianus*, horticulturists treat it as a species on account of its primrose colour as distinct from the striped or spotted colour of *G. quartinianus*. It was discovered in the Usagra Mountains in South Africa, in 1887 by Mr. J. T. Last, who sent corms to Kew where it flowered in 1890. Since then it has been sent from the 'Rain Forest' opposite the Victoria Falls by Mr. Francis Fox, Engineer of the Bridge over the Zambesi. He introduced it into cultivation and innumerable hybrids of *G. primulinus* have now been raised in gardens and nurseries.

*Maiden's Blush* is one such garden hybrid of *G. primulinus* whose other parent it is impossible to trace. It was raised in 1926 at Wisley by the Gladiolus Society.

*G. byzantinus* Bankin is a hardy native of the Mediterranean region. Like all the other species it is always propagated by offsets although it is not completely seed-sterile. It does not, however, set seed in English gardens.

*G. dracocephalus* Hook. is a native of Natal at the foot of the Drakensberg and the upper part of Tugela river. It was first introduced into cultivation by Mr. Thomas Cooper in South Africa.

**Mitosis:** All the species possess prochromosomes which have been fully traced in *G. tristis*, which has the smallest chromosome number,  $2n = 30$ .

Prochromosomes, more properly chromocentres, were first described by Rosen (1892) as deeply staining bodies present in the resting nucleus, distinct from the nucleoli, and by Rosenberg (1904-1909) as corresponding in number to the chromosomes of a species. It has now been definitely shown by Doutreligne (1933) and others that these bodies consist of proximal parts of the chromosomes on either side of the centromere. Among recent workers Smith (1934), Manton (1935) and Raghavan (1938) have confirmed the findings of Doutreligne from their studies on *Impatiens*, *Biscutella laevigata*, and *Polanisia trachysperma* and *Gynandropsis pentaphylla*, respectively. Heitz (1929, 1932, 1935) is of the opinion that sometimes the chromosomes in prophase and telophase show excessive condensation at certain points which stain deeply in aceto-carmine but not in Feulgen. These deeply staining regions contain 'heterochromatin' and are genically inert, the rest of the chromosome being euchromatic.

Grégoire (1932) and Doutreligne (1933) stated that chromonemata are not present in plants with prochromosomes. Smith (1934) merely remarks that fundamentally the chromosome cycle in species with prochromosomes is the same as in large-chromosome species. This is shown to be true in the present paper.

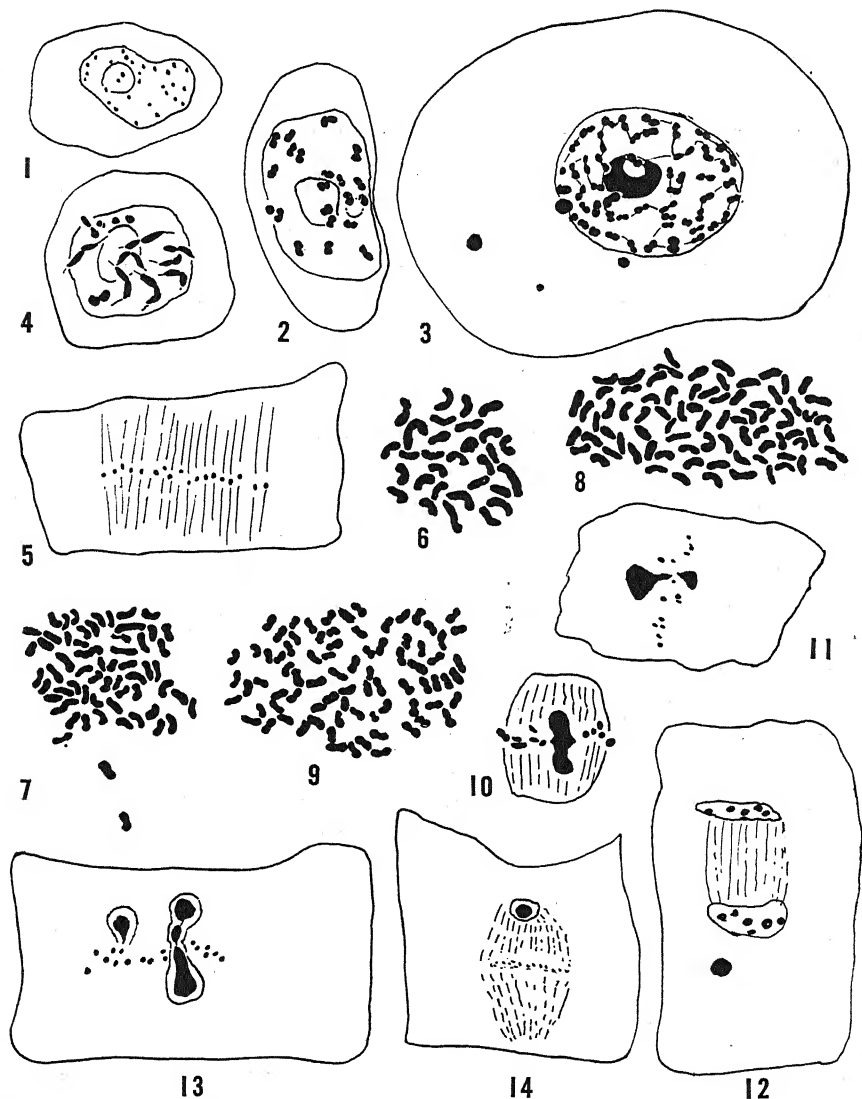
**Prophase-metaphase:** Fig. 1 represents a resting nucleus from the root-tip of *G. tristis* with 30 chromatic bodies uniformly distributed in the nuclear sphere and corresponding to the chromosome number of the species. In Fig. 2, representing early prophase, the prochromosomes appear double while in fig. 4 they show fine filamentous processes becoming chromatic, on either side of these double bodies. The chromosomes are longer than at metaphase, fig. 6.

Although no spirals could be seen, it is quite conceivable that during the mitotic cycle, the chromosomes obey the same laws of condensation and elongation as do the large chromosomes of *Allium* and others. That protein molecules spiralise has been discussed elsewhere (Mensinkai 1939a). Spiralisation takes place both within and between the chromomeres. The chromatic substance being the same in all chromosomes, namely chromatin (protein + nucleic acid) it is not at all unlikely that condensation and elongation of the chromosomes take place by spiralisation and despiralisation. On this basis the chromosomes in interphase will be longest, lying spread out uniformly in the nucleus, and it is only near the centromere that they remain chromatic. These regions appear as chromocentres. As prophase begins, the elongate threads begin to shorten and at the same time become chromatic. The chromatic regions on either side of the spindle-fibre attachment-region being small in the beginning, the chromocentres appear single at this stage. The first indication of chromaticity spreading over the length of the chromosome-arms is in the doubleness of the chromocentres. The filaments in Fig. 4 merely mean chromosome threads in the process of shortening and becoming chromatic. By metaphase, condensation is maximum. Figs. 6, 7; 8, 9 are metaphase plates of *G. tristis*, *G. primulinus*, and *G. dracocephalus* respectively. The chromosome numbers of *G. byzantinus* and *Maiden's Blush* appear to be the same as in *G. primulinus*  $2n = 60$ . In Fig. 8 which belongs to *G. dracocephalus*, the chromosomes are 79 instead of 80 as in Fig. 9, on account of the lagging of the chromosomes in anaphase as shown in Figs. 27, 28.

The nucleolus regularly persists in all species. Figs. 10–26 show one or more nucleoli in several stages of division and migration in these species. It has been suggested (Mensinkai 1939b) that the nucleolus probably bears a charge at the time of its accumulation at the nucleolus-organiser. After its accumulation, it presumably loses its charge, being neutralised by the opposite charge of the organiser. This seems most probable, for in all species, the nucleolus in metaphase-anaphase, either passes to one pole bodily or divides, almost always unequally (Figs. 11, 13–16, 19–20). Its division and migration to the pole are presumably attributable to the stretching



of the spindle. Sometimes two or three nucleoli pass to the same pole as in Figs. 13, 17, 18, 21, 22. The nucleoli are easily distinguished from the chromosomes by their large size and a clear halo encircling them.



*G. tristis* figs. 1-6 except fig. 3 which is *G. primulinus*. Fig. 1. Showing 30 pro-chromosomes. Figs. 2-4. Prophase. Appearance of dumb-bell shape in fig. 2 (cut nucleus) and chromatic connections in fig. 3. Note the elongation of chromosomes and partial disappearance of the filaments in fig. 4. Also mark four nucleolus-like bodies in the cytoplasm in fig. 3. Fig. 5. Compound spindle. Figs. 6-9 metaphase plates. *G. tristis* fig. 6,  $2n = 30$ , *G. primulinus* fig. 7,  $2n = 60$ ; *G. dracocephalus* fig. 9,  $2n = 80$ . Note  $2n = 79$  in fig. 8 of *G. dracocephalus*. Fig. 10. Nucleolus persisting and dividing in metaphase *G. tristis*. Figs. 11-12, *G. byzantinus*. Nucleolus persisting to anaphase-telophase. Figs. 13-14, *G. primulinus*  $2n = 60$  showing the nucleolus dividing and passing to the poles.

The spindle itself is compound. Fig. 5 shows the karyolymph stretched out on either side of each chromosome. These 'fibres' are almost parallel and are presumably produced by an anisotropic condition of the spindle substance round about the chromosome. Later their parallel disposition alters to a bipolar orientation, probably under the influence of pole determinants (Figs. 14, 16, 18, 20).

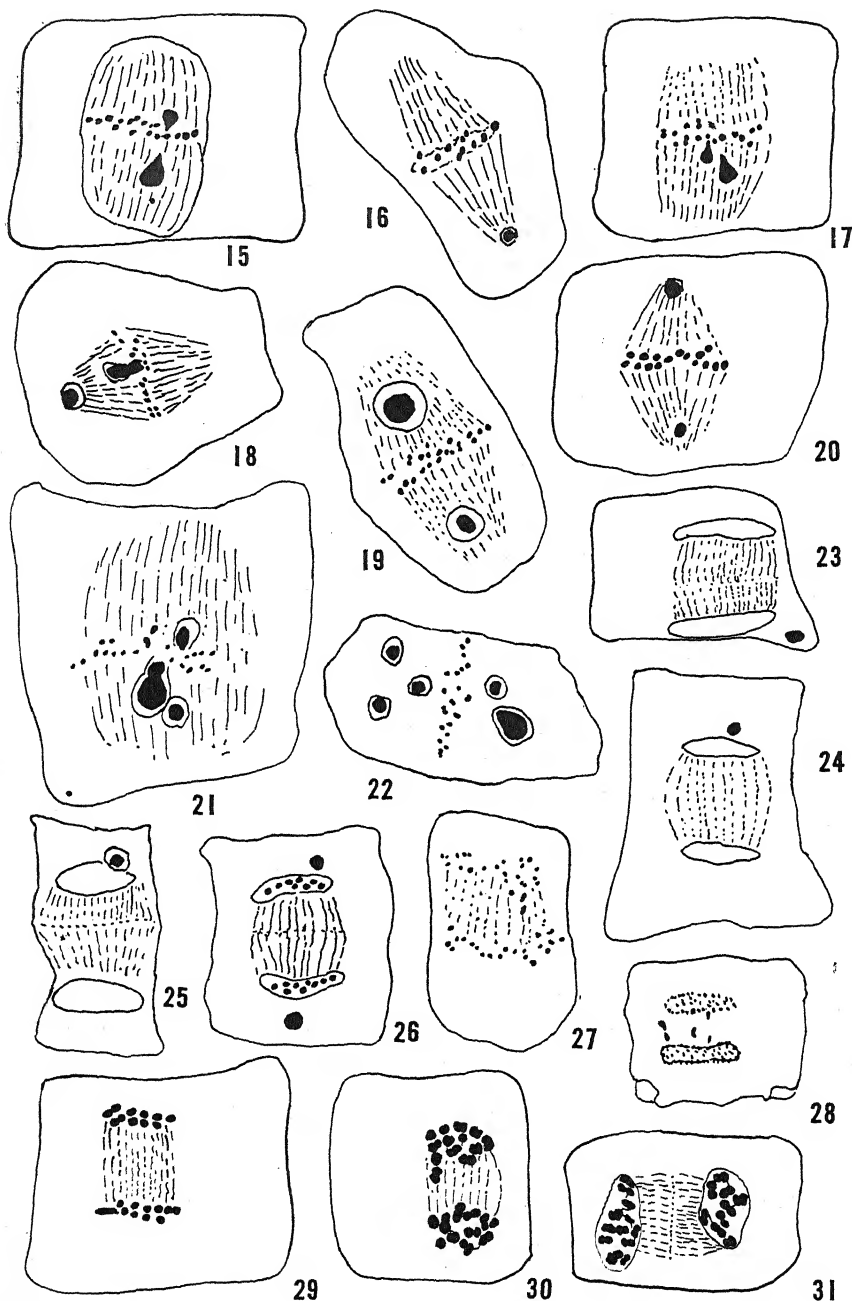
**Anaphase:** The metaphase chromosomes are so uniformly thick in the middle that the constrictions are scarcely visible. After the division of the centromere the daughter chromosomes pass to the poles under the influence of several forces discussed for *Allium* (Mensinkai 1939a). On reaching the poles the chromosomes presumably lose all their surface charge so as to clump to form the tassement polaire, Fig. 29.

**Telophase:** Following this clumping is hydration of the nucleus and a presumable change in pH, which produces an electric charge on the chromosomes. They, therefore, repel and move apart from each other and away from the centre to the periphery. Repulsion is more manifest here than in *Allium* owing to the small size of the chromosomes. This repulsion was first observed and its explanation suggested by Gates (1909). At first the chromosomes appear like single globules in close approximation (Fig. 29), but in later stages their doubleness as dumb-bell shaped bodies is evident (Figs. 30-33). This dumb-bell shape was observed in the telophase chromosomes of *Oenothera* (Gates 1912). As elongation proceeds, the chromosomes touch by their ends. In contrast to *Allium* and other large chromosome genera, the chromosomes never fill the nucleus. In *Gladiolus* the nucleus is large and "hollow" filled with nuclear sap. The repulsive force seems sufficiently strong to drive the chromosomes to the periphery. It is probably the peripheral disposition due to repulsion and the simultaneous elongation that bring in contact the ends of the small centric chromosomes and give an appearance of a meshwork. Smith (1934) figures and describes double processes on either side of the prochromosomes, which coming in contact with others, produce the network appearance. He further regards them as evidence of the duality of the anaphase chromosome. No such double filaments were seen in the chromosomes of *Gladiolus*. It seems natural they should appear single, since they merely represent elongating arms of the chromosomes with the twin chromonemata closely intertwined. The network appearance is only an optical image of the filamentous prochromosomes peripherally distributed over the nuclear sphere, and simulates a criss-cross arrangement when viewed vertically.

The chromosomes become more and more achromatic as they elongate. Figs. 29–34 clearly picture the gradual migration of the chromosomes to the periphery and the transformation of a chromatic meshwork from a state of double to single chromatic bodies with achromatic filamentous connections. Loss of chromaticity proceeds from the ends towards the centromere, so that the chromosomes come to take on a dumb-bell shape in later stages. These double bodies are the prochromosomes and they do not all necessarily remain visible in interphase. Progressive achromaticity seems to reduce their dumb-bell shape to a globular shape, which on further loss of stainability may leave the nucleus perfectly transparent without any prochromosomes. Whenever visible in interphase, they do not, therefore, necessarily correspond to the chromosome number. However, they will not exceed that number. Even Feulgen reaction did not seem to show all of them nor all the chromatic threads, although it is expected to reveal chromatin substance. The threads are extremely fine and they as well as some of the chromocentres probably fall below the limits of visibility. However, Fig. 3 in early prophase (*G. primulinus*  $2n = 60$ ) seems to show the slightly chromatic meshwork and about 56 prochromosomes getting dumb-bell shaped. In prophase the chromosomes begin to shorten and the Feulgen reaction shows itself without difficulty.

In *G. dracocephalus*, an unexpected abnormality was observed—a somatic bridge in anaphase-telophase in the root-tip (Fig. 35). This is similar to those described in *Allium margaritaceum* and *A. giganteum* (Mensinkai 1939c).

A most interesting feature is the persistence of the nucleolus to late anaphase and in some cases to interphase. This was observed in both *G. primulinus* and *G. dracocephalus*. In *G. tristis* the nucleolus generally persists only up to metaphase. Figs. 23–26, show the nucleoli persisting to late anaphase, and in telophase. The nucleoli in anaphase are so far away from the daughter nuclei (Figs. 23, 24) that they will not in all probability be included in them. Fig. 3, from the root-tip of *G. primulinus* stained in Feulgen-Light Green, shows two large and two small nucleolus-like bodies in the cytoplasm. It seems certain that they represent nucleolar material both from their viscosity and their green colouration exactly similar to the nucleolus within the nucleus. They have either survived the last division and been left out of the daughter nuclei so as to lie in the cytoplasm, or have arisen from the lipid material of the cytoplasm during cell-metabolism. The second suggestion is less probable as in that case they would be seen in many more nuclei and in greater abundance. These observations, therefore, are quite



Figs. 15-28, *G. dracocephalus*. Figs. 15-24, showing the numbers of nucleoli and stages in their division in metaphase-anaphase. Figs. 25-26, Telophase with persisting nucleoli lying in the cytoplasm. Figs. 27-28, Anaphase-telophase with lagging chromosomes. Figs. 29-31, *G. tristis*, stages leading to prochromosomes; 29-30, late anaphase. Fig. 30, anaphase, the chromosomes slightly apart and appearing constricted; 31, telophase chromosomes clearly constricted and further apart.

in harmony with the hypothesis proposed before (Mensinkai 1939b) that the nucleolus is mainly made up of lipides and acts partly as a fuel substance in the cell-metabolism. The nucleolus in the cytoplasm might well behave as a storage product, like fat in animals.

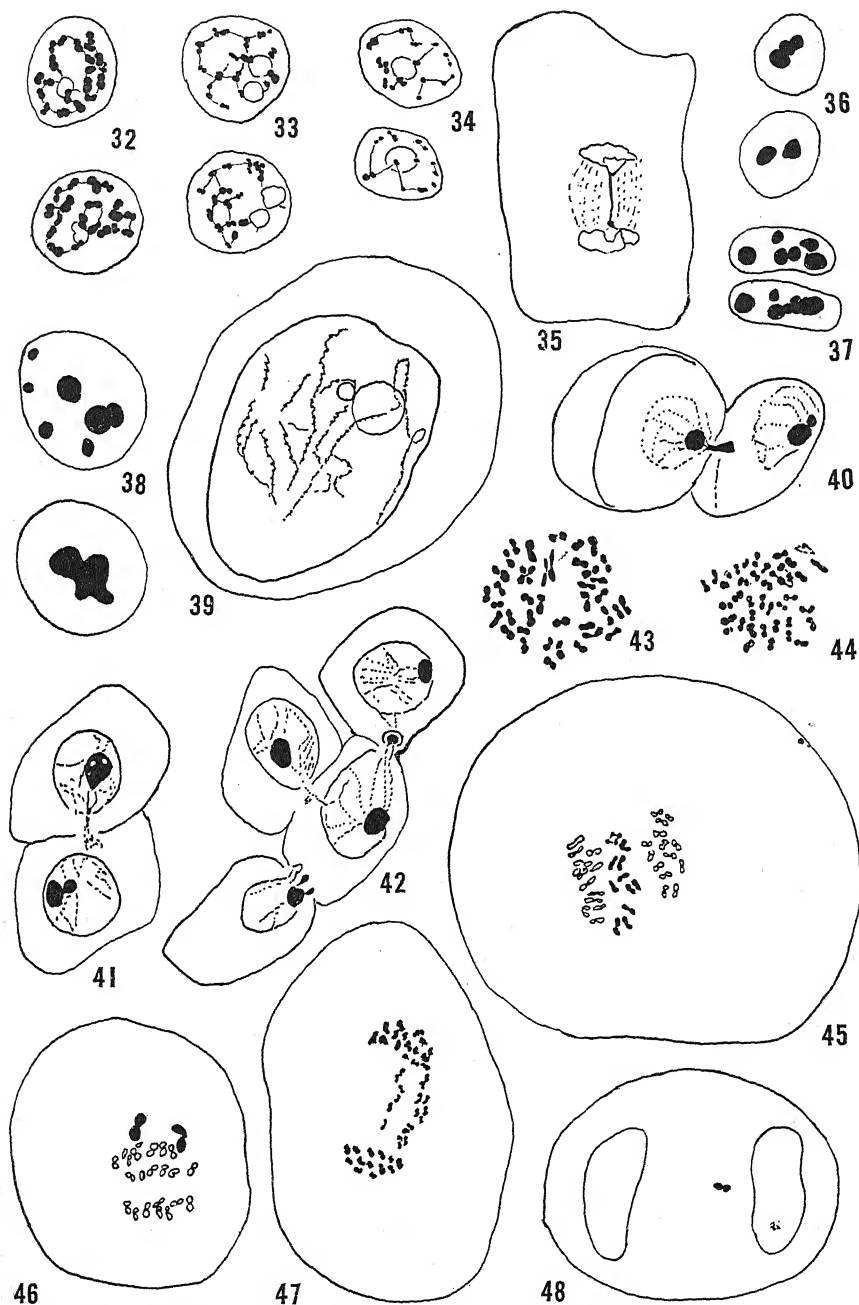
**Meiosis:** As in somatic mitosis, nuclear division in meiosis is seldom seen. Most of the pollen mother cells are in resting stage. The haploid number of *G. dracocephalus* is 40. Figs. 39, 43, 44, represent pachytene, prometaphase and metaphase respectively of *G. dracocephalus*. In prometaphase (Fig. 43) all are bivalents, showing that the species is an allopolyploid. However, in metaphase I a chain of three bivalents could occasionally be seen (Fig. 44).

Except in *G. tristis*, pachytene frequently showed cytomixis in all the species and hybrids, especially *Maiden's Blush* (Figs. 40-42). Cytomixis was first observed and described by Gates (1911) in *Oenothera gigas* and subsequently by several others, e.g. Church (1929) in grasses, Kattermann (1933), Kihara and Lilienfeld (1934) and Bhatia (1938), in *Triticum* hybrids. Church (1929) distinguishes three types of cytomixis. In the first it persists to pachytene (spireme stages), in the second it lasts to diplotene-diakinesis, and in the third it is abundant in diakinesis.

The explanation of this phenomenon (Gates 1911) as depending on differences in hydrostatic pressure in the contiguous cells is quite reasonable. It is quite recognised that cells are interdependent in their activity and have intercellular protoplasmic strands connecting them. When they are in contact and when the nucleus also lies near the ectoplast, the difference in pressure in the cells showing such contact may result in protrusion of the chromosome threads of one nucleus into the cytoplasm of the other cell.

Whether these ejected threads retract during condensation (by spiralsation) in later stages cannot be said with certainty. The fact that the phenomenon has been classified into three types depending on the stage of its persistence suggests that such a retraction is possible. It must, however, depend on the nature and amount of extension of the chromosome threads. If they show no clumping or folding as in Fig. 41 they may be pulled back into the proper nucleus, mainly by condensation. If a mass of chromatin is shot out as in Figs. 40, 42 it is quite unlikely that it gets drawn back into the nucleus. The chromatin-lump (Fig. 42) is obviously too big to pass through the openings in the cell wall. In such cases cytomixis is definitely disadvantageous to the species on account of its pathological nature.

It has been suggested by Church (1929) and others that cytomixis is a characteristic of hybrids. This view is partly supported



Figs. 32-34. *G. tristis*, telophase-interphase. Note clearly dumb-bell shaped chromosomes in Fig. 32 getting single and globular in Figs. 33-34 and the chromatic filaments becoming achromatic and disappearing in Fig. 34. Fig. 35, *G. dracocephalus*, anaphase bridge. Figs. 36-37 Maximum number of nucleoli in telophase in *G. tristis* and *G. primulinus* respectively. Fig. 38. *G. dracocephalus*. Maximum number of nucleoli in telophase. Fig. 39-48, P.M.C. *G. dracocephalus* except Figs. 40 and 42 which belong to *G. primulinus* and Maiden's Blush respectively. Fig. 39. Pachytene. Figs. 40-42. Cytomixis at pachytene. Figs. 43-44. Prometaphase and metaphase I. Note a chain of three bivalents in Fig. 44. Figs. 45-48. Anaphase I and telophase I.

by *Maiden's Blush* showing the phenomenon very frequently. Figs. 40, 41, 42, which belong to *G. primulinus*, *G. dracocephalus*, and *Maiden's Blush* respectively give an idea of the frequency of cytomixis in the species. It remains to be seen if cytomixis has any genetical significance, but chromatin losses which are not lethal for the cell might clearly lead to the functioning of a nucleus with different genetic properties.

In addition to this behaviour, the species are characterised by irregular chromosome-distribution owing to multivalent formation, lagging of chromosomes and double non-disjunction of bivalents. Fig. 44 shows metaphase I of *G. dracocephalus* with a chain of three bivalents, Fig. 45, represents early anaphase I with several half bivalents and one bivalent lying on the equator; Figs. 46, 47 show double non-disjunction and lagging of chromosomes in anaphase I. Fig. 48 represents a lagging half-bivalent in *G. dracocephalus*. It will be seen from Fig. 43 that bivalents predominate over the multivalents. *G. dracocephalus*, therefore, is probably an allopolyploid. Although meiosis of *G. tristis* could not be studied, it appears to be a polyploid from the occasional occurrence of three nucleoli in the nucleus (Fig. 36). Similarly the maximum number of nucleoli to be seen in *G. primulinus* was 6 (Fig. 37). In *G. dracocephalus*, however, it was not possible to see more than 6-7 (Fig. 38) despite the fact that a higher number of nucleoli is to be expected from its chromosome number ( $2n = 80$ , Fig. 9). From the nucleolar and chromosome evidence, therefore, *G. tristis* seems to be a triploid, *G. primulinus* a hexaploid and *G. dracocephalus* an octoploid, and the basic number for the genus will be 10, not 15. The related genera as well, e.g. *Iris*, *Musa*, *Tritonia* and *Dierama* have 10 or its multiples as the most frequently recurring number.

### Discussion

All the species of *Gladiolus* propagate mainly vegetatively by offsets and bulbs although most of them are facultatively sexual. The percentage of seed formation varies from species to species.

A large number of factors have been associated with this type of reproduction. The chief of them may be said to be (1) irregularities in sporogenesis either of microspore or megaspore or both (2) high polyploidy (3) hybridisation. All these factors appear to be operative in the genus, although it is difficult to be precise as to which is preponderant.

**Irregular Meiosis:** Defective sporogenesis seems largely responsible in causing sterility. Irregular meiosis brings about segre-

gation of chromosomes different from what is present in normal tetrads. In *G. dracocephalus* the anomalies in meiosis, though less frequent, are multivalent formation, lagging of chromosomes and non-disjunction of bivalents. This leads to the production of some amount of functionless pollen.

**Polyploidy:** It has been a fact of observation that vegetatively reproducing species have, as a rule, higher chromosome numbers than their sexual relatives, e.g. the popular roses. Polyploidy, therefore, seems to favour vegetative reproduction. With autopolyploids the reason seems fairly obvious. A too great likeness of the chromosomes is a serious cause of irregularity, as is a too great dissimilarity. Autopolyploidy therefore, always leads to multivalent formation and irregular assortment. Allopolyploids, however, form regular bivalents although occasionally giving rise to multivalents, and their meiosis is less irregular.

Of the four species, *G. dracocephalus* has a maximum chromosome number and should, therefore, be sexually more sterile than *G. primulinus* or *G. byzantinus*. *G. tristis* is a triploid and behaves like an odd-numbered polyploid with inherent irregularity of meiosis.

**Hybridisation:** Several workers, Winge (1917), Täckholm (1920-22), Ostenfeld (1921), Turesson (1930), advocate hybridisation as a primary cause of chromosomal aberrations. Winge (1917) emphasized the importance of deficient synapsis. When two specifically different chromosome sets of like number are brought together, they may sometimes act in complete harmony at all stages of meiosis and mitosis. If the chromosome compatibility is less they synapse poorly or not at all. This results in irregular distribution of chromatin for the spores. The truth of this theory is apparent from the sexual sterility so common in hybrids.

It must also be mentioned that sexual sterility may well be the result of nutritive disturbance through abnormal cultural conditions. In both *G. primulinus* and *G. dracocephalus* it was very often noticed that most of the pollen sacs were half empty with dead cells, in later stages, although at early stages they appeared filled with pollen mother cells. This might be due to the cold climate.

Although mutation cannot be excluded, irregular meiosis, polyploidy and hybridisation, have played a recognisable role in producing vegetative reproduction in the genus. Species reproducing only vegetatively are genetically stable in that the variations produced are stored up. The hybrid chromosome complements and the same external characters are maintained. They are clones. Contrary to this conclusion, *Gladiolus* shows innumerable forms and varieties. This seems solely due to retention of sexual fertility which ensures



retention of crossing over, responsible for the process of segregation and, therefore for variation. The species do not behave like pure clones but they are 'sub-sexual', the sexual character being less apparent but genetically effective.

### Summary

Four species of *Gladiolus* and one variety have been cytologically examined. The chromosome numbers of these species have been confirmed and of *G. dracocephalus* determined for the first time as  $2n = 80$ .

The behaviour of prochromosomes in *G. tristis* ( $2n = 30$ ) and *G. primulinus* ( $2n = 60$ ) has been fully examined. It is shown that the mitotic cycle in species with prochromosomes is essentially the same as in species with large chromosomes.

The nucleolus regularly persists to metaphase and sometimes until interphase. This is regarded as supporting the interpretation that the nucleolus, consisting of lipoids (fats), may partly act as a fuel substance.

The spindle appears to be compound in structure.

Cytomixis has been described in the genus for the first time.

The basic number is shown to be 10, not 15 as previously reported.

The causes and effects of vegetative reproduction in the genus are briefly discussed.

### Acknowledgment

I heartily thank Professor R. Ruggles Gates for his kindly advice, helpful criticism and constant encouragement in the course of this work.

I also thank the Navelgund Sirsangi Trust for offering me a grant to study in the University of London.

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## Chromosome Numbers in Plumbaginaceae

By

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For years I have been interested in determining the meiotic chromosome numbers in this family. Among the genera in this family I have specially investigated *Statice* and *Armeria* which were little known karyologically.

Materials have been sent me from the botanical gardens of Lisbon, Madrid and Paris, and to the authorities of these institutions I wish to express my most appreciative thanks. As a fixing fluid our familiar Farmer's fluid was chiefly used with the young flower buds. They were cut about  $14\ \mu$  thick and stained with Heidenhain's iron-alum haematoxylin.

In general these materials may be overstained, so that I have stained them for a day.

### *Statice*

I previously studied 5 species, namely *St. incana*, *St. latifolia*, *St. sinuata*, *St. spicata* and *St. Suworowii* and found their meiotic chromosome number to be eight. An account of the same has already been published. We have now five other species which have also eight or its multiples of the meiotic chromosomes as mentioned below.

*Statice bicolor*. This plant (Jap. name Tōgōsō), growing near Port Arther, Dairen, Kirin etc., has large rose flowers.

It has eight ellipsoidal meiotic chromosomes. The pollen mother cells are very large ( $18\ \mu$  in diam. at IM) in spite of the small size of flowers.

*Statice echioides*. There are 16 V-shaped meiotic chromosomes in the second metaphase.

The chromosome arrangement agrees with Thomson's arrangement of corpuscles in stable equilibrium (11-5).

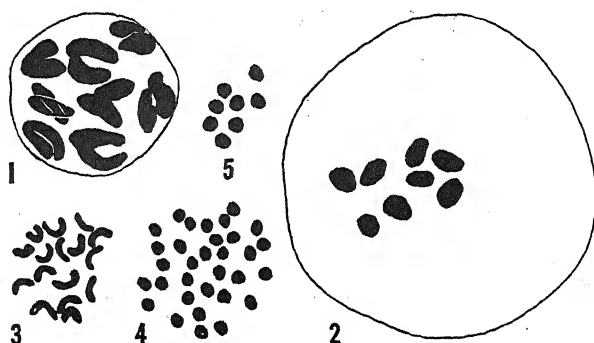
From this it is clear that even the V-shaped rods can be arranged in the same way as spherical chromosomes.

*Statice globulariaefolia*. This has 32 meiotic chromosomes which are neatly arranged in 15-11-6.

The 6 chromosomes in the centre may possibly be rearranged into 5-1.

The pollen mother cells are as large as those of *St. bicolor*.

*Statice Bonduelli*: It has 8 gemini as shown in Fig. 1.



Figs. 1-5. *Statice*. 1. *St. Bonduelli*, diaphase. 2. *St. bicolor*, IM. 3. *St. echinoides*, IIM. 4. *St. globulariaefolia* IM. 5. *St. dictyoclada*, 1A.  $\times 3000$ .

*Statice dictyoclada*. It has 8 meiotic chromosomes which are equally spherical. The pollen mother cells are very large;  $25\mu$  in diam. in the first anaphase.

Generally the pollen mother cells in this genus are much larger than those of *Armeria*.

The chromosome numbers in *Statice* were first found by Aleskowsky (1930) to be 18 or its multiples in the somatic cells in several plants which have not yet been touched by the present writer. In 1937 Wulff published a paper with figures in *Jahrbücher f. wiss. Botanik*, saying that he had found 36 somatic chromosomes in *St. limonium*, 18 in *St. sinuata* and 9 meiotic chromosomes in *St. Suworowii* and affirming that the basic number in *Statice* was 9. But Aleskowsky's and Wulff's results unfortunately did not coincide with mine,—that is, the meiotic chromosome numbers counted by the present writer in *St. sinuata* and *St. Suworowii* were 8 each.

In the chromosome accounts of these already published the meiotic figures are so clear that it is almost impossible to miscount the chromosomes.

In *St. Suworowii* it is interesting to see that not only are the pollen mother cells arranged breadth-wise in a line, but they also show the same anaphasial stage of nuclear division. Generally this phenomenon is rather rare. In most cases the karyokinetic stages of the pollen mother cells in the same anther are diverse, especially in the extreme cases even the first anaphasial figures and tetrads are often found coexisting.

### Armeria

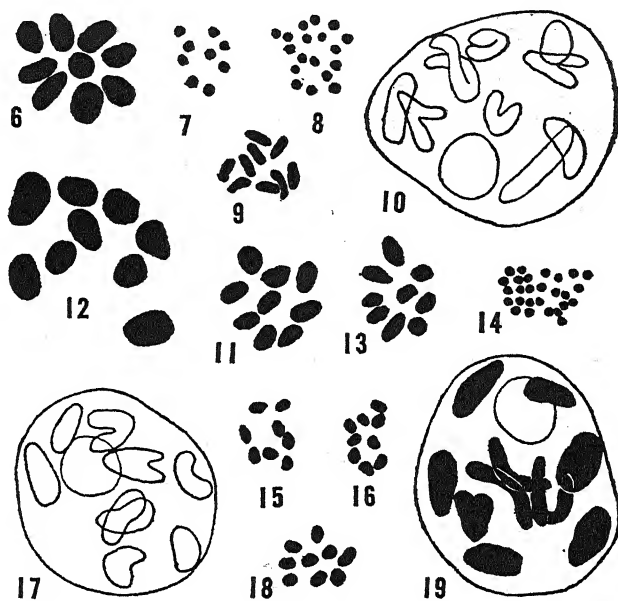
Lately the writer has counted the meiotic chromosomes in 15 species of *Armeria* as follows:—

<i>Armeria alliacea</i>	9	<i>Armeria elongata</i>	9	<i>Armeria plantaginea</i>	9
<i>allioides</i>	9	<i>filicaulis</i>	9	<i>pubescens</i>	9
<i>alpina</i>	18	<i>majellensis</i>	9	<i>sardoa</i>	8
<i>canescens</i>	9	<i>mauritanica</i>	27	<i>splendens</i>	9
<i>denticulata</i>	9	<i>pinifolia</i>	8	<i>Welwitschii</i>	9

The above numbers of meiotic chromosomes determined by the figures of diaphase, IM, IA, IIM and IIA, were already published.

Among them *A. alliacea* and *A. filicaulis* have large meiotic chromosomes, while *A. pinifolia* has small ones in the first metaphase. In the second metaphase, likewise, *A. canescens* has large short rod-shaped chromosomes while *A. allioides* has small granular ones.

The chromosome arrangement similar to that of Thomson's corpuscles in stable equilibrium is clearly observed in *A. alpina* and *A. mauritanica*, the former being 11-6-1, the latter 13-10-4.



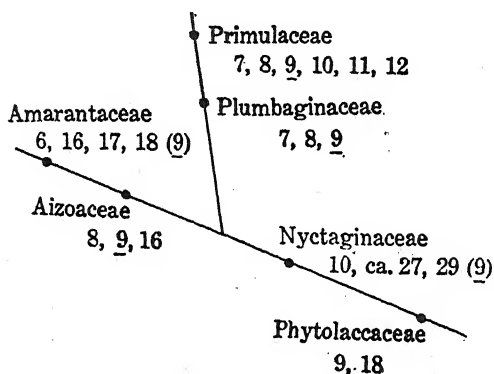
Figs. 6-19. *Armeria*. 6. *A. alliacea*, IM. 7. *A. allioides*, IIM. 8. *A. alpina*, IIA. 9. *A. canescens*, IIM. 10. *A. denticulata*, Diaphase. 11. *A. elongata*, IM. 12. *A. filicaulis*, IA. 13. *A. majellensis*, IA. 14. *A. mauritanica*, IA. 15. *A. pinifolia*, IM. 16. *A. plantaginea*, IIM. 17. *A. pubescens*, Diaphase. 18. *A. splendens*, IA. 19. *A. Welwitschii*, Diaphase.  $\times 3000$ .

*Armeria* chromosomes were first counted by Fernandes (1931). He and Mac Turk (1934) found the meiotic chromosome number in *A. maritima* to be 7. Tischler (1937) has also admitted this number to be basic in *Armeria*. But from our studies the basic number in *Armeria* should be 9 instead of 7, for our species of *Armeria* which have been investigated have 9 or its multiples, except *A. pinifolia* and *sardoa*, which have 8.

Quite recently Griesinger (1938) also counted 9 meiotic chromosomes in 7 species of *Armeria*. Thus almost a half of the species in this genus have 9 meiotic chromosomes. Our meiotic chromosome number 10, which was found in *A. vulgaris* some years ago, seems to have been derived from 9. Thus, we now know that the basic chromosome number in *Armeria* is definitely 9. Fernandes' 7 is not the basic number.

### Phyletic relationship in Plumbaginaceae on the basis of chromosome numbers

Putting together the above we now know that three basic numbers are in this family: 7, 8 and 9. The first was found in *Plumbago capensis* by Dahlgren (1916), the second in *Statice* and the third in *Armeria* by the present writer. According to the result of sero-diagnostic studies by F. Malligson the Plumbaginaceae lies between



Primulaceae and Aizo-Nyctaginaceae. Now the former has a basic number 9, and both the latter also 9; thus it can be also concluded from the chromosomic point of view that the Plumbaginaceae stands closely allied to Primulaceae and Aizo-Nyctaginaceae, as shown in the adjoining diagram.

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**Appendix :** After this article had been sent to the press, there appeared a paper by H. M. Phillips on the karyology and phyletic relationships of the Plumbaginaceae (Chronica Botanica Vol. 4, No. 4/5). He reported the chromosome numbers in 41 species of *Armeria*. Most of my previous determinations in this genus agree with his results. Also his conclusion, that the basic chromosome number in Plumbaginaceae is 9, and this family is closely related to Primulaceae is the same as mine.

# The Cytology of Autotetraploid Kale, *Brassica oleracea*

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Received April 5, 1939

## Contents

	Page
1. Introduction . . . . .	77
2. Material and Methods . . . . .	77
3. General Cytology of Autotetraploid Kale . . . . .	78
1. Diakinesis . . . . .	78
2. 1st Metaphase . . . . .	78
3. 2nd Metaphase . . . . .	78
4. Fertility of Autotetraploid Kale . . . . .	81
5. Secondary Associations . . . . .	82
1. Diploid . . . . .	82
2. Tetraploid . . . . .	83
6. Summary . . . . .	86
7. References . . . . .	87

## 1. Introduction

The cytology of autotetraploids is now quite well understood e.g. Upcott (1935) on autotetraploid *Lycopersicum esculentum*; Randolph (1935) on autotetraploid maize. The data given in this paper are very similar to those reported by Upcott (1935) and are not therefore discussed at any length. In addition the frequency of secondary associations in diploid and autotetraploid kale have been examined.

## 2. Material and Methods

Two distinct autotetraploid kale stocks have been examined. The first was a tetraploid obtained by the callus method of somatic doubling. This was of the thousand-head variety. Sister diploid cuttings were also obtained. Secondly an autotetraploid was obtained from one embryo of a double-seed of marrow-stem kale. The sister embryo was diploid.

The cytological preparations were permanent smears of pollen mother cells. They were mostly fixed in La Cour 2 BE, but also weak Flemming, La Cour 2 B and a fixative described by Catcheside (1934) were tried. 2 BE appeared to be the most satisfactory fixative, but Catcheside's may give better results at diakinesis. All slides were stained by Newton's iodine gentian violet method.

Drawings were made with the help of a camera lucida at a magnification of 5,000 and have been reduced to 2/3 in reproduction.

### 3. The General Cytology of Autotetraploid Kale

The chromosome number of *Brassica oleracea* is  $n=9$  and  $2n=18$ . The cytology of the diploid has been described by Richharia (1937) and with special reference to secondary associations by Catcheside (1937).

#### 1. Diakinesis.

Figs. 5 & 6 are drawings of cells of tetraploid kale at diakinesis. A variable number of quadrivalents is formed, see Table 1. The frequency of the different types of quadrivalent is given in Table 2. The latter results are very similar to those of Upcott (1935). Two univalents were also seen in two cells.

At least three types of chromosomes can form quadrivalents. Two of these types are chromosomes with a median centromere, one type being considerably larger than the other. The third type is a chromosome which has one arm much shorter than the other.

#### 2. Metaphase 1 and Anaphase.

These were not examined in any detail. In polar view it is possible to recognise quadrivalents and also secondary associations, see Fig. 4.

In side views of metaphase 1 some types of quadrivalent are very obvious; other types would be difficult to distinguish from secondarily associated bivalents. A considerable number of the ring quadrivalents were found orientated as shown in Figs. 8, 9 & 10. Such an orientation tends to cause two of the chromosomes to be left on the equator at anaphase. In other cases a 3:1 segregation seems probable, see Figs. 8 & 9. The zig-zag arrangement of rings, see Fig. 12, which is a diagram not a figure, was not observed. It must also be stated, however, that it would be one of the types confused with secondarily associated bivalents. A trivalent and univalent were also observed in one cell.

Occasional lagging chromosomes and division of univalents were seen at first anaphase. Some of the dividing 'univalents' may be chromosomes which were members of a quadrivalent at metaphase.

#### 3. Metaphase 2 and Anaphase.

Between first and second divisions there is an interphase stage during which the chromosomes are arranged around the periphery









Table 1. Frequency of quadrivalent formation at diakinesis

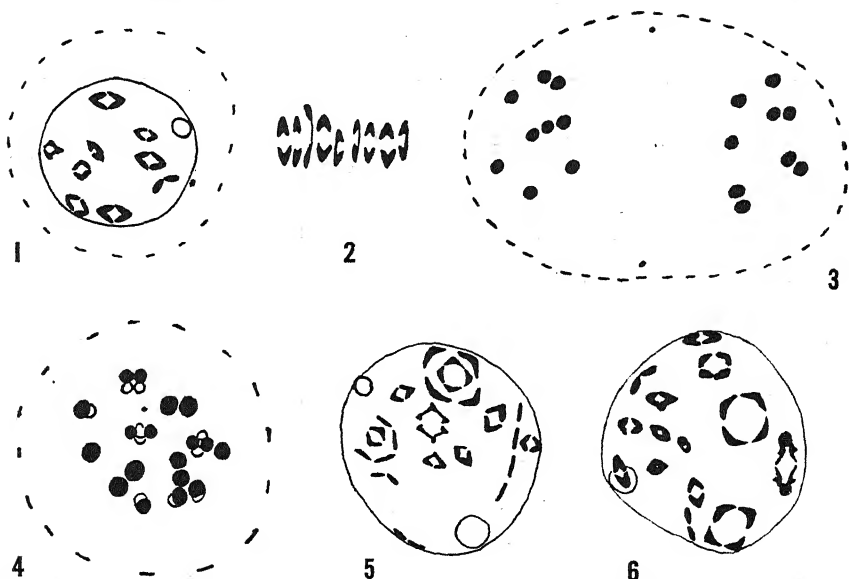
Number of quadrivalents per cell	0	1	2	3	4	5	6	7	8	9	Total
Number of cells	0	0	1	2	3	4	0	0	0	0	10

Mean number per cell = 4.0. 2 cells in these 10 also had 2 univalents each.

Table 2. Frequency of different types of quadrivalent at diakinesis

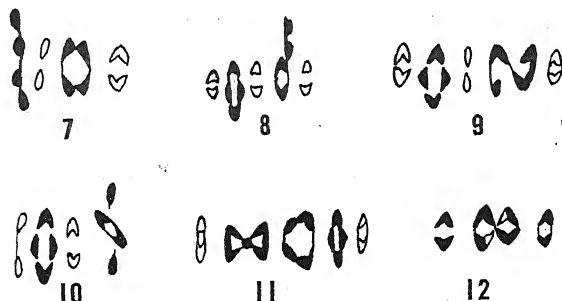
Type of quadrivalent	Number found	% occurrence	cf. % occurrence in <i>Lycopersicum</i> <sup>1)</sup>
	22	55	51.3
	14	35	34.7
	1	2.5	1.5
	1	2.5	0
	1	2.5	2.1
	1	2.5	2.1
Total	40		

1) Data from Upcott (1935).



Figs. 1-3. Meiosis in diploid kale. 1. Diakinesis. 2. First anaphase. 3. Second metaphase polar views of both plates. Left side,  $1(3) + 1(2) + 4(1)$ . Right side,  $3(2) + 3(1)$ . Figs. 4-6. Meiosis in tetraploid kale. 4. First metaphase polar view, 3 quadrivalents and secondary association of bivalents. 5. Diakinesis, 5 quadrivalents. 6. Diakinesis, 4 quadrivalents.

of the nucleus, see Catcheside (1937). The chromosomes are equidistant from each other as at diakinesis. In the tetraploid all traces of quadrivalent formation disappear. The two chromosomes of a ring quadrivalent, as in Fig. 7, going to the same pole and



Figs. 7-11 & 12. Meiosis in tetraploid kale. 7-11. Side views of quadrivalents at first metaphase, neighbouring bivalents in outline to show orientation. 12. Diagram, not figure, to show 'zig-zag' orientation, which was not seen.

attached to each other have become entirely separate. I did, however, find one nucleus where two chromosomes did appear to be joined. This nucleus is shown in Fig. 13. The two attached chromosomes were probably joined by an interstitial chiasma at first division.

Second metaphases have been examined to obtain information of two kinds, (a) frequency of numerically irregular disjunctions at first anaphase, and (b) the frequency of secondary associations. This latter topic is discussed later.

Table 3. Frequency of plates with 20, 19, 18, 17 or 16 chromosomes at second metaphase

Preparation	Number of plates with chromosomes							Total plates
	21	20	19	18	17	16	15	
1. Temporary aceto-carmin a.	0	2	5	20	5	1	0	33
2. " " b.	0	0	2	8	2	0	0	12
3. Permanent slide 2 of " 24.5.	0	1	5	6	3	1	0	16
4. " " 1 of 26.5.	0	0	1	5	1	0	0	7
5. " " 3 of 27.5.	0	2	4	32	15	3	0	56
Total	0	5	17	71	26	5	0	124
% occurrence	0	4	14	57	20	4	0	

The frequency of plates with the regular chromosome number of 18 and the irregular numbers of 19, 17 etc. due to irregular disjunctions at first anaphase is given in Table 3. Numerically irregular disjunctions appear to be very frequent. This is probably due to the small size of the chromosomes favouring the discordant (Darlington, 1937, page 131) 'diamond' type of orientation. It is not due to non-terminalisation of the chiasmata making the configurations more rigid.

In a few second metaphase polar views small chromosomes could be seen. These are halves of univalents which have divided at first division. At second anaphase some of these divide again.

#### 4. The Fertility of Autotetraploid Kale

Autotetraploid kale is very much less fertile than the diploid. The diploid of the thousand-head stock was self-compatible to a certain degree. It did not, however, set as many seeds per fruit when it was selfed as when it was crossed. The autotetraploid from this same stock was also self-compatible.

It can be seen from Table 4 that the seed fertility of the autotetraploid is about 35% of that of the diploid. It may be that the high percentage of numerically irregular disjunctions at meiosis will

Table 4. Fertility of diploid and autotetraploid kale

A=no or very small development of ovule.  
S+s=shrivelled seeds of several sizes.  
G=good seeds.

	A	S+s	G	Total ovules	%G
(a) Diploid selfs					
1.	281	7	212	500	42.2
2.	255	4	222	481	46.3
3.	171	7	127	305	40.1
(b) Diploid by diploid of another stock	80	3	278	361	77.0
(c) Tetraploid selfs					
1.	203	39	55	297	18.5
2.	306	15	72	393	18.2
3.	455	36	72	563	12.8
(d) Tetraploid female × diploid male	71	27	39	137	28.5

The fertility of the autotetraploid is thus about 15/42 of that of the diploid, i.e. 36% approx. of the diploid.

account for the whole of this infertility. It is not possible with the data so far obtained to suggest how the infertility is produced, e.g. the proportion of pollen and embryo-sacs which are inviable, the proportion of zygotes which are inviable, whether less ovules are fertilised in the tetraploid than in the diploid. It appears that pollination of the tetraploid with the diploid may help to solve the problems—in this case all the pollen has a balanced constitution.

The percentage of good pollen in the autotetraploid is about 96%. This is similar to the percentage found in autotetraploid *Datura*, see Blakeslee and Cartledge (1926), but not similar to autotetraploid *Lycopersicum esculentum* where there is about 25%

bad pollen, (Jørgensen, 1928). I also examined autotetraploid *L. esculentum* plants in 1938 and found that there was about 25% bad pollen as compared with 2% in the diploid.

## 5. Secondary Associations

### 1. Diploid *Brassica oleracea*.

Catcheside (1937) has discussed very fully the occurrence and significance of secondary associations in diploid *B. oleracea*. He found very good evidence for suggesting that the basic number was six and that the haploid set could be written as AA BB CC D E F. The maximum association is then 3(2) + 3(1), i.e. 3 associations of 2 chromosomes each and 3 chromosomes not in any associations. Also the frequency of occurrence of plates with the different number of associations, 1(2), 2(2), or 3(2), fitted a binomial distribution.

Table 5. Secondary associations in diploid kale at second metaphase (present investigations)

Type of plate	Numbers of plates seen
9(1)	3
7(1)+1(2)	19
5(1)+2(2)	23
3(1)+3(2)	14
1(1)+4(2)	1
6(1)+1(3)	9
4(1)+1(3)+1(2)	16
2(1)+1(3)+2(2)	5
1(3)+3(2)	0
3(1)+2(3)	1
5(1)+1(4)	3
3(1)+1(4)+1(2)	2
Total	96

Catcheside did, however, find associations other than those to be expected from the constitution of the haploid set suggested above, e. g. associations of three chromosomes, or 4(2) in the same plate. He concluded that these associations were not due to bad fixation or to the possibility of mistaken observation, but were caused by real structural complexity, i.e. the simple formula given above for the constitution of the haploid set is too simple.

My own observations and those of Richharia (1937), see Table 6, only agree with those of Catcheside numerically when associations of two are considered. Both Richharia and I found a

much higher frequency of associations of three than Catcheside, see Table 6. Although the number of plates examined by Richharia and myself is very much less than the number examined by Catcheside, there is no doubt that our observations do not agree with his on this point.

It does not seem probable that the difference between Catcheside's and my results is due to the personal factor in deciding what is and what is not an association of three chromosomes. The personal factor might be important if associations of four chromosomes were being considered—it is very easy sometimes to suggest that these are two associations of two. There was no reason for suggesting

Table 6. Secondary associations in diploid *Brassica oleracea*  
(a) All plates.

Material	Total plates	No. of plates 9(1)	No. of plates with (2)'s only	No. of plates with (3)'s (and (2)'s)	No. of plates with (4)'s (and (2)'s)
Catcheside (1937) M1.	385	32 = 8.3%	305 = 79.2%	39 = 10.1%	5 = 1.3%
Catcheside (1937) M2.	215	11 = 5.1%	168 = 78.2%	33 = 15.3%	1 = 0.5%
Richharia (1937)	49	2 = 4.1%	18 = 36.7%	19 = 38.3%	7 = 14.4%
Present paper. M2.	96	3 = 3.1%	56 = 58.3%	31 = 33.4%	5 = 5.3%

(b) Plates containing (2)'s only.

Material	No. of plates which were				Total plates with (2)	Total of assoc	Mean assoc/cell
	9(1)	1(2)+7(1)	2(2)+5(1)	3(2)+3(1)			
Catcheside (1937) M1.	32 = 9.5%	103 = 30.6%	122 = 36.2%	80 = 23.7%	337	587	1.74
Catcheside (1937) M2.	11 = 6.2%	48 = 26.8%	78 = 43.6%	42 = 23.4%	179	330	1.84
Richharia (1937)	2 = 10.0%	6 = 30.0%	6 = 30.0%	6 = 30.0%	20	36	1.80
Present paper. M2.	3 = 5.1%	19 = 32.2%	23 = 39.0%	14 = 23.7%	59	107	1.81

In part (b) of the Table only those plates to be expected from the simple formula AA BB CC D E F are considered. All three investigations give very similar results in this part.

that the plates examined in this investigation were badly fixed. The difference is therefore a real one and may be due to either environment or to a difference in genetical constitution of the two sets of material. Moffett (1934) found that the degree of secondary pairing found in pears is not so great as that found in apples by Darlington and Moffett (1930):

## 2. Tetraploid *B. oleracea*.

Even assuming the simple formula AA BB CC D E F for the constitution of the haploid set, the diploid set of *B. oleracea* is AAAA BBBB CCCC DD EE FF, so that in the tetraploid a plate showing 3(4) + 3(2) might be found. We should expect to find associations of six since in the diploid associations of three were quite common.

Secondary associations in the tetraploid were examined at second metaphase. The occurrence of quadrivalents at first metaphase makes observations of secondary associations at this stage of little value. Compared with the diploid, observations are more difficult to make since the chromosomes are more crowded on the plate. There are twice the number of chromosomes on a plate only

about one and a half times the area of the diploid. Also as has been previously suggested there does appear to be a personal factor in deciding sometimes whether four chromosomes are in one association

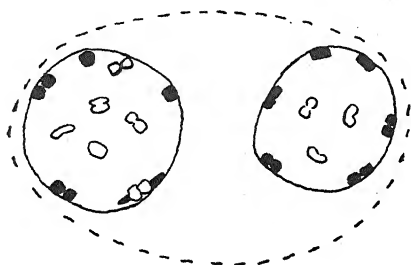


Fig. 13. Cell containing two interphase nuclei in tetraploid kale. Only a few of the chromosomes drawn. Nucleus on the left contains at 5 o'clock body which consists of two chromosomes and is due to two chromosomes of a quadri-valent not separating.

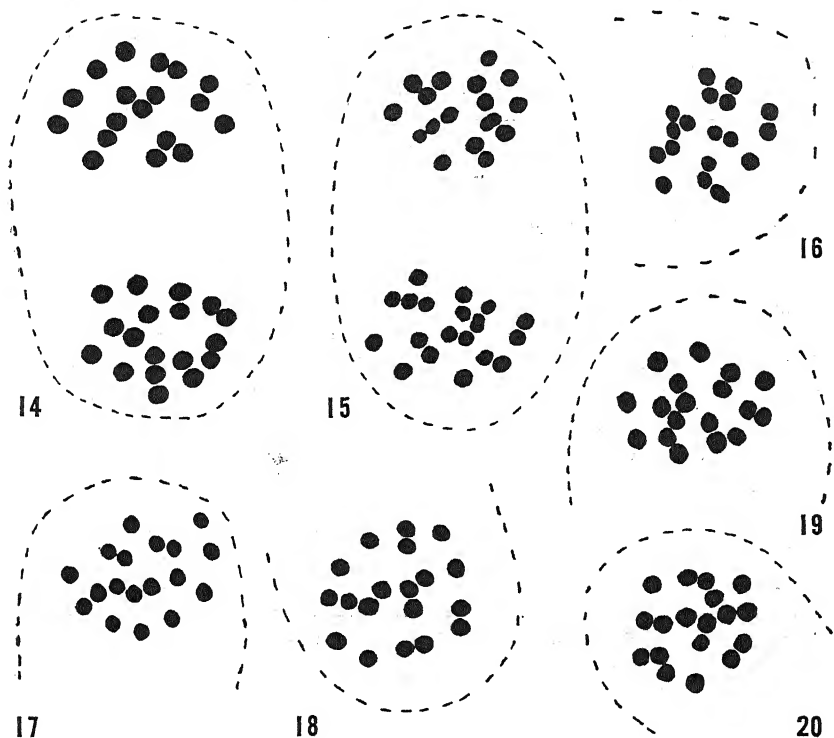
of four or in two associations of two each. This type of difficulty is illustrated in the figures.

As has been previously stated there is an interphase stage between first and second divisions. During interphase the chromosomes are found on the periphery of the nucleus more or less equidistant from each other. The secondary associations observed at second metaphase are therefore true secondary associations and do not represent continuations of

primary associations formed at first division.

Figs. 14-20 show secondary associations in second metaphase plates of tetraploid kale, and Table 7 gives a summary of the associations observed in 56 plates. It can be seen from the table that the hypothetical maximum of  $3(4) + 3(2)$  was not observed. Plates in which only two chromosomes were not in any associations i.e. almost the maximum association were, however, found in 8 cells. As was to be expected there is great variation in the numbers of associations of 4, 3, and 2 found in different plates. It will also be noticed that associations of more than four chromosomes are observed quite frequently. This was to be expected since associations of three were common in the diploid. Associations of five are also to be expected since some of the plates with 18 chromosomes will be of the type  $2n + 1 - 1$  from irregular disjunction at first division. The common occurrence of associations of more than four in the tetraploid is additional evidence for considering associations of three in the diploid not to be artefacts.

The frequency of secondary associations observed are such as would be expected if the formation of an association depends upon similar chromosomes being adjacent to each other at prometaphase as suggested by Catcheside (1937). A calculation similar to that made by Catcheside for the diploid, depending upon the number of neighbours and non-neighbours possessed by each chromosome, has been made to find what is the expected frequency of occurrence of



Figs. 14-20. Polar views of second metaphase in tetraploid kale. 14. Both plates in polar view, 18:18. 15. Both plates in polar view, 19:17. 16-20. One plate only in polar view.

Secondary associations are very marked in all plates.

Fig. 20 is an example of a difficult plate, it might contain 1(8), but this (8) might be 2(3)+1(2).

The associations in the plates are

Fig. 14, top 2(3)+3(2); bottom 1(3)+5(2).

Fig. 15, top 1(4)+2(3)+1(2); bottom 1(5)?+1(3)+3(2).

Fig. 16 1(5)+1(4)+1(3)+2(2). Fig. 17 1(5)+2(2).

Fig. 18 1(4)+1(3)+3(2). Fig. 19 1(6)+1(3)+2(2).

Fig. 20 1(8)+1(3)+2(2), or 3(3)+3(2).

associations of 4, 3, and 2 respectively. Unfortunately one has to make several more assumptions than appear in Catcheside's calculation for the diploid. This makes the calculation of little value and it is not given in this paper.

It can, however, be pointed out that the frequency of occurrence of secondary associations in the tetraploid is what would be expected from the hypotheses made by Catcheside (1937). It is also to be noted that the occurrence of secondary associations does depend upon the chance of chromosomes being adjacent to each other and the maximum association must therefore be rare. The attempt of

Table 7. Secondary associations in tetraploid kale at second metaphase

Each vertical column represents one plate, thus the first column shows a plate with 1(5)+1(3)+10(1).

Assocn.	20 plates with 18 chromosomes																				Total
(1)	10	5	7	2	2	5	2	2	3	6	4	2	6	4	4	7	4	7	2	3	87
(2)			3	3	1	1	5	2	4	3	3	2	4	5	5		2	4	4	1	52
(3)	1	3		2	3		2	2	1	2	1	4				1		1	1	3	27
(4)		1		1					1					1	1	1				1	7
(5)	1		1		1	1				1							2		1		8
(6)						1		1													2
(7)																					0
(8)																1					1

Assocn.	15 plates with 18 chromosomes															Total	Grand Total
(1)	4	4	5	3	3	2	3	4	5	5	4	2	2	5	4	54	142
(2)	4	3	1	3	4	4	3	3	3	5	2	5	1	3	3	47	99
(3)	2	1	1	1	1	1	3	1	1	1	2	2	2	1	1	21	48
(4)			2		1				1		1		2	1		8	15
(5)		1				1		1						1		4	12
(6)				1												1	3
(7)																0	0
(8)																0	1

Assocn.	5 plates with 19 chromosomes										Total
(1)	11	4	4	3	5						27
(2)	4	5	4	2	3						18
(3)			1	1							2
(4)			1	1	2						4
(5)	1		1								2
(6)											0
(7)											0
(8)											0

Assocn.	14 plates with 17 chromosomes														Total
(1)	5	1	5	5	4	3	5	3	6	7	3	4	2	5	56
(2)	3	4	1	1	1	4	2	2	4	1	1	1	1	1	27
(3)	2	1	2	2	1	2		2	1		1	1	1	2	18
(4)			1	1	2		2	1		2	1	2	1	1	14
(5)	1									1					2
(6)											1				1
(7)															0
(8)															0

Haga (1938) to carry genome analysis a stage further in the genus *Brassica* by observing secondary associations in the allopolyploid species is therefore of doubtful value.

## 6. Summary

1. Autotetraploid kale forms from 2 to 5 quadrivalents per cell at diakinesis, with a mean of 4 per nucleus.



2. Only 57% of the second metaphase plates contained 18, the balanced number of chromosomes.

3. The seed fertility of autotetraploid kale is about 35% of that found in the diploid.

4. Secondary associations of chromosomes were examined in the diploid and in the tetraploid.

(a) A high frequency of associations of three chromosomes was found in the diploid. This was not found by Catcheside (1937). This difference between the two sets of material appears to be a real one and is either genetical or environmental.

(b) At second metaphase in the tetraploid, there are very many different types of plates containing different numbers of associations of 4, 3, and 2 chromosomes. This is to be expected from the fact that if the haploid set can be represented as AA BB CC D E F, then the diploid set in the tetraploid is AAAA BBBB CCCC DD EE FF.

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## Cytogenetical Studies in *Avena*. II On the progenies of pentaploid *Avena* hybrids\*

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About 10 years ago it was found by the present author that pentaploid *Avena* hybrids revealed striking irregularities in the meiotic behavior of chromosomes (NISHIYAMA 1929). For example, in the  $F_1$  hybrids ( $2n = 35$ ) between *A. barbata* ( $n = 14$ ) and *A. fatua* ( $n = 21$ ), 2–11 bivalents including some chromosome complexes are formed and the other chromosomes are left unpaired. At first anaphase all bivalents divide and move toward the poles as usual. However univalents still remain on the equatorial plate. Then they divide longitudinally, although some of them occasionally fail to divide. In the succeeding stage these halves of univalents travel to the poles and usually become incorporated in the daughter nuclei which are mainly composed of the halves of bivalents. In the second anaphase the halves of bivalents divide in regular fashion, but those of univalents stay for a little while on the equator, and wander very slowly and irregularly to the poles without splitting. Most of these lagging chromosomes arrive at the poles and join the daughter nuclei. Occasionally several chromosomes remain outside forming micro-nuclei. In addition, certain irregularities such as regressions are sometimes observed during the maturation divisions (NISHIYAMA 1929). These cytological results were fairly confirmed by EMME (1932).

The failure of chromosome pairing is the starting point of the abnormalities in gonogenesis; it also causes a high sterility in pentaploid *Avena* hybrids. Accordingly there has been, as yet, no study on the cytogenetics of their offspring.

By repeated attempts, however, I obtained a small number of  $F_2$  seeds from hybrids, *A. barbata* ( $n = 14$ )  $\times$  *A. fatua* ( $n = 21$ ), by selfing and also by back-crossing with *A. fatua*. These supplied a sufficient number of plants of later generations for cytological studies. It is especially noticeable that the mode of chromosome

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\* Contributions from the Laboratory of Genetics, Biological Institute, Kyoto Imperial University, No. 103.

number change in the offspring is quite different from that of triploid *Avena* hybrids (c.f. NISHIYAMA 1934).

Part of the present investigation was briefly reported at the 5th general meeting of the Genetic Society of Japan in 1933. Detailed data of cytogenetical studies will be given in this paper.

### I. Fertility of pentaploid *Avena* hybrids

According to EMME (1929) two pentaploid hybrids of *A. barbata* × *A. diffusa* (*A. sativa*) and of *A. abyssinica* × *A. diffusa* were found to be completely sterile. However, I obtained a few seeds from certain pentaploid *Avena* hybrids (NISHIYAMA 1929). In the years 1928–31, further investigations were extensively made on the seed production of similar interspecific hybrids; the results obtained so far are shown in Table 1. These hybrids usually set

Table 1. Fertility of pentaploid *Avena* hybrids

Hybrids	No. of ears examined	No. of florets examined	No. of seeds	% of seeds	Year
<i>A. barbata</i> × <i>sativa</i>	23	3018	0	0.00	1929
"  "	26	3100	25	0.81	"
"  "	6	682	0	0.00	1930
"  "	3	283	0	0.00	1931
Reciprocal	15	1856	0	0.00	1930
<i>A. barbata</i> × <i>fatua</i>	18	2469	6	0.24	1928
"  "	35	5486	169	3.08	"
"  "	9	1616	3	0.19	1929
"  "	5	621	0	0.00	1930
Reciprocal	4	611	5	0.82	"
<i>A. barbata</i> × <i>sterilis</i>	18	1268	0	0.00	1928
"  "	31	2434	4	0.16	"
<i>A. barbata</i> × <i>byzantina</i>	20	1526	0	0.00	1929
"  "	18	1322	0	0.00	"
<i>A. sterilis</i> × <i>barbata</i>	6	534	0	0.00	1930
<i>A. abyssinica</i> × <i>sativa</i>	9	469	0	0.00	"
<i>A. abyssinica</i> × <i>fatua</i>	12	1551	0	0.00	"
<i>A. abyssinica</i> × <i>sterilis</i>	12	996	0	0.00	"

The mark "\*" shows the open-pollination.

no seeds at all by selfing, but only on the hybrid of *A. barbata* × *fatua* a small number of seeds were produced very sporadically. In the open-pollination, however, some of the hybrids showed a very low production of seeds. The degree of the fertility appears to be much affected by field conditions and also by the differences in  $F_1$  combinations. When these sterile hybrids were grown in the neighbourhood of hexaploid oats, not di- or tetraploids, they occasionally bore fully developed seeds, most of which might result from natural cross-pollination. By artificial back-crossing with *fatua*-pollens, the writer also obtained some viable seeds from the hybrid, *A. barbata* × *fatua*.

For examining pollen grains in the present case, the same method as shown in my previous article was followed (NISHIYAMA 1934). Well filled pollen grains were counted as good, while those which were empty or reduced in content were classified as bad. Table 2 summarizes all data on pollen fertility in  $F_1$  hybrids and their parental species.

Table 2. Pollen fertility in pentaploid *Avena* hybrids and their parental species

Plant	Total number of pollens examined	Number of good pollens	% of good pollens	Year
<i>A. barbata</i> ( $n = 14$ )	553	550	99.46	1930
<i>A. abyssinica</i> ( $n = 14$ )	501	472	94.21	"
<i>A. fatua</i> ( $n = 21$ )	524	520	99.24	"
<i>A. sativa</i> ( $n = 21$ )	582	574	98.63	"
<i>A. sterilis</i> ( $n = 21$ )	587	580	98.81	"
<i>A. byzantina</i> ( $n = 21$ )	613	603	98.37	"
<i>A. barbata</i> $\times$ <i>fatua</i>	1015	184	18.13	"
<i>A. barbata</i> $\times$ <i>sativa</i>	724	72	9.94	1929
"	1049	127	12.11	1930
<i>A. barbata</i> $\times$ <i>sterilis</i>	571	40	7.01	1929
"	1114	26	2.33	1930
<i>A. barbata</i> $\times$ <i>byzantina</i>	681	42	6.17	"
<i>A. abyssinica</i> $\times$ <i>fatua</i>	514	56	10.89	"
<i>A. abyssinica</i> $\times$ <i>sativa</i>	677	41	6.06	"
<i>A. abyssinica</i> $\times$ <i>sterilis</i>	914	62	6.78	"

As is evident from the table the pure species are characterized by excellent fertility, in most cases showing more than 98% of good pollen grains. On the contrary, all of seven  $F_1$  combinations give a markedly low percentage of good pollen grains, i.e. from 2 to 18%. From these observations it can be concluded that the production of no seeds, or but few, in pentaploid *Avena* hybrids is due mainly to the gametic, especially pollen abortion.

## II. Cytological observations

(1)  $F_2$ - $F_5$  generations. From the hybrid, *A. barbata* ( $\varphi$ )  $\times$  *fatua* ( $\sigma$ ), five  $F_2$  plants were obtained in 1929-30. In the following year (1931) the reciprocal hybrid produced two  $F_2$  segregates also. The chromosome numbers and meiotic pairing in these plants were studied at first metaphase in PMC (Table 3). They had 42-67 chromosomes, the majority having many more chromosomes than the parental species with the higher chromosome number. In all of the plants, the numbers of paired and unpaired chromosomes were so variable as it was difficult to determine what the normal configuration of chromosomes should be. The number of bivalents shown in Table 3 is the highest number so far observed in any of the segre-

gates<sup>1)</sup>. Since some univalents frequently mated with bivalents and formed trivalents, a smaller number of univalents than that described in the table were occasionally found. Besides, the occurrence of chromosome complexes such as quadri- or higher polyvalents was not seldom. It was also remarkable that different plants with the same diploid chromosome number should usually exhibit a difference in the amount as well as the variability of their chromosome pairing.

Table 3. Chromosome numbers in the progenies from *A. barbata*  $\times$  *fatua*

Plant number	Chromosome numbers of segregates			
(F <sub>2</sub> )	F <sub>2</sub>	F <sub>3</sub>	F <sub>4</sub>	
29-300-1	48 = 19 <sub>II</sub> + 10 <sub>I</sub>	59 = 27 <sub>II</sub> + 4 <sub>I</sub> + f	56 = 28 <sub>II</sub> + 0 <sub>I</sub>	
-2	55 = 24 <sub>II</sub> + 7 <sub>I</sub>	59 = 28 <sub>II</sub> + 3 <sub>I</sub>	59 = 28 <sub>II</sub> + 3 <sub>I</sub>	
-3	64 = 29 <sub>II</sub> + 6 <sub>I</sub>	60 = 28 <sub>II</sub> + 4 <sub>I</sub>	60 = 28 <sub>II</sub> + 4 <sub>I</sub>	
-4	67 = 30 <sub>II</sub> + 7 <sub>I</sub>	61 = 27 <sub>II</sub> + 7 <sub>I</sub>	61 = 28 <sub>II</sub> + 5 <sub>I</sub>	
30-301b-1	64 = 30 <sub>II</sub> + 4 <sub>I</sub>	62 = 30 <sub>II</sub> + 2 <sub>I</sub>	61 = 29 <sub>II</sub> + 3 <sub>I</sub>	
		64 = 30 <sub>II</sub> + 4 <sub>I</sub>	62 = 29 <sub>II</sub> + 4 <sub>I</sub>	
		65 = 30 <sub>II</sub> + 5 <sub>I</sub>	63 = 30 <sub>II</sub> + 3 <sub>I</sub>	
			64 = 31 <sub>II</sub> + 2 <sub>I</sub>	
31-306*-1	42 = 17 <sub>II</sub> + 8 <sub>I</sub>	14 = 7 <sub>II</sub> + 0 <sub>I</sub> (const.)		
-2	56 = 24 <sub>II</sub> + 8 <sub>I</sub>			

\* 31-306 represents the offspring of *A. fatua* (♀)  $\times$  *barbata* (♂).

For example, a plant (31-306-1) had the same chromosome number as *A. fatua* ( $2n = 42$ ), but there were never seen 21<sub>II</sub> but 17<sub>II</sub> + 8<sub>I</sub> in which 1-4 trivalents were often formed. Fig. 1 represents some metaphase plates in PMC from F<sub>2</sub> plants possessing 55 and 64 chromosomes. As can be seen in this figure, many bivalents are of the loose, end-to-end type.

In later stages of the meiosis the principal behavior of chromosomes was seen to be nearly similar to that of pentaploid F<sub>1</sub> hybrids of *Avena* (NISHIYAMA 1929).

Some F<sub>3</sub> plants were grown from a 67-chromosome F<sub>2</sub> (29-300-4), and the chromosome number of 7 segregates were counted at first metaphase in PMC. As given in Table 3, all of these plants possessed a smaller number of chromosomes than the parent i.e., in general they showed a reduction in the number of univalents and in some cases a further reduction in bivalents (Fig. 1, d,e.) Of course there was also found a wide variation in the chromosome pairing from plant to plant and also in one and the same individual.

Eight F<sub>4</sub> segregates derived from an F<sub>3</sub> (30-430-8) with 65-chromosomes had 56-64 chromosomes (Table 3). In the single 56-chromosome plant (31-552-19), 28 bivalents could be found, but

1) The same is true in the other tables.

individual pollen mother-cells frequently showed the formation of chromosome complexes or the failure of pairing in two or more bivalents. From these meiotic irregularities it is readily understood why its single progeny, examined cytologically, had 55 chromosomes,  $27_{II} + 1_I$ .

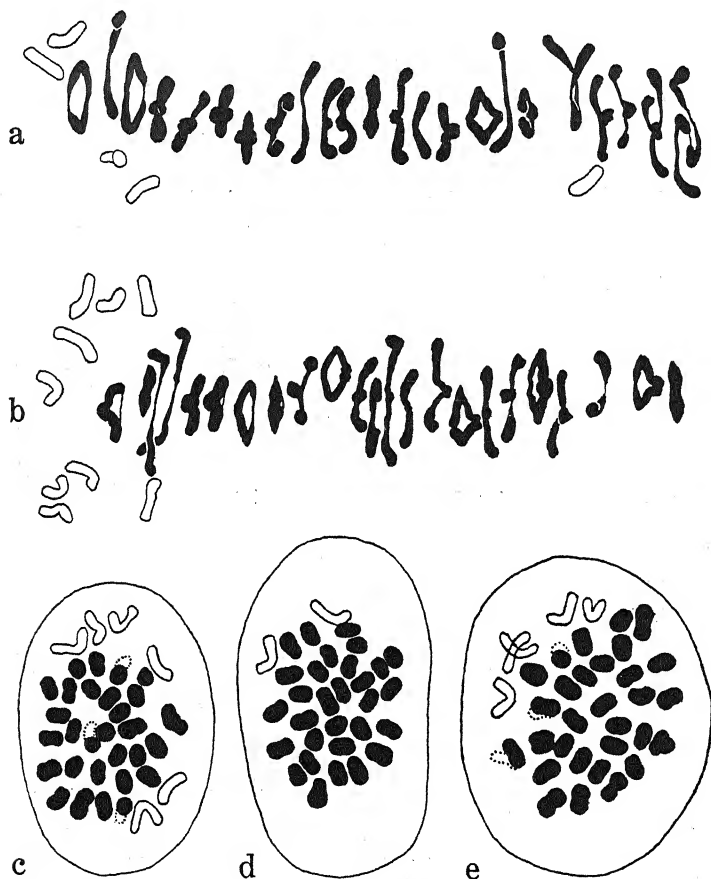


Fig. 1. Chromosomes at first metaphase of PMC in the selfed progenies of *A. barbata*  $\times$  *fatua*. a,  $F_2$  plant (29-300-2),  $1_{IV} + 2_{III} + 20_{II} + 5_I = 55$ . b, The same plant,  $23_{II} + 9_I = 55$ . c,  $F_2$  plant (30-301b-1),  $29_{II} + 6_I = 64$ . d,  $F_3$  plant (30-430-2),  $30_{II} + 2_I = 62$ . e,  $F_3$  plant (30-430-8),  $30_{II} + 5_I = 65$ .

An  $F_2$  plant of *A. fatua*  $\times$  *barbata*, 31-306-1, having  $17_{II} + 8_I$  set two seeds by self-pollination. They germinated, but only one seedling grew to maturity. As previously reported, it was quite unexpected to discover that this plant had only seven bivalents paired closely while its gonogenesis followed the regular fashion (NISHIYAMA 1933). In the following generations, the newly raised diploid plant produced some strains showing different characters

but the chromosome number in all was invariable. The new diploids were readily crossed with the known diploid *Avena* species and the  $F_1$  hybrid proved to have the normal fertility. It was also cytologically determined that the new diploids had the same genom constitution, AA, as diploid species of *Avena* (NISHIYAMA 1933).

(2) Back-crossed progenies. In 1928-29 there were secured four  $BF_1^{1)}$  plants from *A. barbata*  $\times$  *fatua* by back-crossing with *fatua*-pollen. As shown in Table 4, two  $BF_1$  had 49 chromosomes and the others 52 and 54 chromosomes respectively (Fig. 2, a). At first metaphase the maximum number of bivalents was found to be 20-23 while the remainder were usually left as univalents. Some modifications of the chromosome conjugation were also frequently met with, as in  $F_2$  progenies described above (see Table 6).

Six  $BF_2$  plants with 42-44 chromosomes were the offspring of a 49-chromosome  $BF_1$  (Table 4). They had 20-21 bivalents and

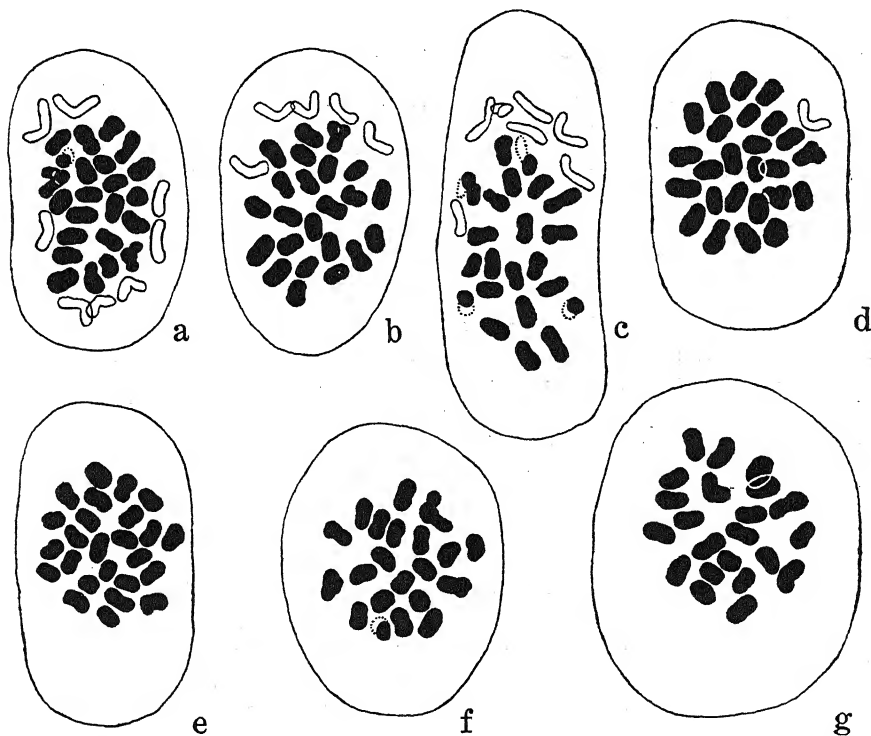


Fig. 2. Chromosomes at first metaphase in PMC of back-crossed progenies. a,  $BF_1$  (28-290),  $23_{II}+8_I=54$ . b,  $BF_2$  (29-390-58),  $23_{II}+5_I=51$ . c,  $BF_2$  (29-390-56),  $22_{II}+7_I=51$ . d,  $BF_3$  (30-467-11),  $25_{II}+1_I=51$ . e,  $BF_4$  (31-557-1),  $25_{II}=50$ : f,  $BF_4$  (32-509-7),  $21_{II}=42$ . g,  $BF_5$  (33-601-7),  $22_{II}=44$ .

1) The symbol indicates the offspring of  $F_1$  which is back-crossed with *A. fatua*.





The progenies of six BF<sub>2</sub> and five BF<sub>3</sub> differing in chromosome numbers were grown in succeeding years. In these progeny, the chromosomal situation was nearly like that in BF<sub>2</sub> (Table 4, Fig. 2, d-f). It should be especially noted that there were obtained three kinds of hyperhexaploid BF<sub>3</sub> having 22<sub>II</sub>, 24<sub>II</sub> and 25<sub>II</sub>, although some chromosomes sometimes, even frequently, fail to mate. Table 5 represents the frequency of occurrence of unpaired chromosomes in

Table 5. Frequency of PMC with univalents which fail to pair in hexa- and hyperhexaploid derivatives

Hybrid derivatives	0	1	PMC with 2 3 4 univalents			5	6	Total	% of PMC with univalents
BF <sub>2</sub> (n = 21 <sub>II</sub> )	214		1					215	0.43
BF <sub>4</sub> (n = 21 <sub>II</sub> )	240		15					255	5.88
BF <sub>4</sub> (n = 22 <sub>II</sub> )	229	2	41			2		274	16.42
BF <sub>4</sub> (n = 24 <sub>II</sub> )	227	14	36	1	6			284	20.07
BF <sub>4</sub> (n = 25 <sub>II</sub> )	113	31	40	11	11		1	207	45.41

these BF<sub>3</sub>-BF<sub>4</sub> progenies. One or three univalents seen in some pollen mother-cells may not be all there were in that cell, owing to their being obscured by bivalents at first metaphase. More-over, it was certain that such cases were frequently accompanied by the formation of a trivalent. Fig. 2, e and g, show the chromosomes at first metaphase in PMC of BF<sub>4</sub>-BF<sub>5</sub> derivatives from these hyperhexaploids. In BF<sub>5</sub> I further found two other descendants with 25<sub>II</sub> the chromosomal situation of which was also very similar to that described just above. From these cytological results it can be said that euhexaploid progenies show the most regular pairing of chromosomes, while in hyperhexaploids the more chromosomes there are, the more frequently the failure of pairings occurs.

In short, in the present hybrids the mode of chromosome number change differs from that of triploid *Avena* hybrids as discussed in my previous article (c.f. NISHIYAMA 1934). The hybrid offspring usually shows an unstable pairing of chromosomes, so that it is not easy to get completely constant plants with hyperhexaploid or higher numbers of chromosomes. The chromosome configuration "21<sub>II</sub>" seems to be the most balanced type and the majority of other configurations appears to have a tendency to return to that configuration.

As can be seen from Table 4, female gametes which produced BF<sub>1</sub> plants possessed 28, 31 and 33 chromosomes. From their meiotic pairings of the chromosomes it is probable that in addition to some extra chromosomes they possess the same genom constitution, ABC, as *A. fatua* (HUSKINS 1928). But in the 28- chromosome gamete, one of these genomes seems to have lost one element, i.e. it contains

6 chromosomes instead of 7. Such gametes might be produced through incomplete regression in which the occasional loss or duplication of some chromosomes occurred as pointed out in my previous paper (NISHIYAMA 1929). In later generations, however, most of the functional gametes, male and female, had 21 or more chromosomes, in which at least 3 genomes A, B and C were usually present.

### III. Fertility and plant-vigour in relation to chromosome numbers

As previously demonstrated the pentaploid *Avena* hybrids show a high percentage of sterility. However, back crossed progenies showed a markedly wide variation in their fertility. Sterility appears to be mainly caused by the abnormal chromosome situation of plants. Therefore seed production and plant-vigour have made the subject of special studies in relation to chromosome numbers and chromosome configurations.

Table 6. Fertility of  $BF_1$

Plant No.	No. of chromosomes	Fluctuation of univalents	Chromosome complexes	No. of ears	No. of florets	% of seeds	Length of culms (cm)	No. of culms
29-370-1	$49=20_{II}+9_I$	8-11	+	3	421	51.31	155	22
-2	$52=21_{II}+10_I$	9-12	+++	4	899	43.49	149	34
-3	$49=20_{II}+9_I$	7-12	+++	4	226	0.00	136	30

+++ one or more tri- or quadrivalents are often found.

++ sometimes found.

+ rarely found.

- no chromosome complex is observed.

As can be seen in Table 6, three  $BF_1$  grew vigorously. Two plants were semi-sterile but the remainder bore no seed at all, although the latter had the same chromosome configuration,  $49=20_{II}+9_I$ , as one of the former.

Concerning the same investigation on 51  $BF_2$  plants all of the data obtained have been summarized in Table 7. The percentage of the seed production was extremely variable, but no plant was observed to be completely sterile. It is hard, therefore to find a definite correlation between fertility and chromosome numbers. However, it may be stated that all plants having 28 chromosome elements (bivalents + univalents), except a few, are usually of high fertility. At present it is quite unknown what combination of chromosomes they have. But it may be said that those with this number are the more favorable for seed production than the others. Working on the theory of the genom it seems very probably that

they possess the fertile combination of chromosomes (KIHARA 1924), i.e. in addition to the same genom constitution, AABBCc, as *A. fatua* there is the B' genom derived from *A. barbata* (AAB'B', NISHIYAMA 1934), and some chromosomes of B' are present in the double doses and the other in the single dose.

Table 7. Fertility of BF<sub>2</sub> (progenies from a BF<sub>1</sub> with 54 chromosomes)

Plant No.	No. of chromosomes	Fluctuation of univalents	Chromosome complexes	No. of ears	No. of florets	% of seeds	Length of culms (cm)	No. of culms
29-390- 11	46=22 <sub>II</sub> +2 <sub>I</sub>	2-4		4	384	83.85	106	62
- 87	47=22 <sub>II</sub> +3 <sub>I</sub>	3-7	+	6	387	41.34	95	43
-105	"	3	-	1	50	46.00	105	28
-101	"	2-7	+	2	168	41.07	125	15
- 43	47=23 <sub>II</sub> +1 <sub>I</sub>	1-3	+	2	66	4.55	94	33
- 59	"	1-5	+	8	342	29.53	71	23
- 30	48=23 <sub>II</sub> +4 <sub>I</sub>	3-6	+	4	277	93.50	138	31
- 18	"	4-6	+	4	269	72.12	117	39
- 77	"	4-6	+	7	545	40.55	123	25
-104	"	4-7		3	266	84.21	112	26
-108	"	4-6	-	3	178	60.67	115	38
- 65	48=23 <sub>II</sub> +2 <sub>I</sub>	2-4	-	3	178	87.64	121	34
-115	48=24 <sub>II</sub> +0 <sub>I</sub>	0-4	+	3	129	4.65	95	13
- 32	49=22 <sub>II</sub> +5 <sub>I</sub>	5-9	-	3	177	44.63	119	24
- 45	"	3-5	+	5	258	61.24	108	52
- 53	"	5-7	-	3	207	33.33	126	39
-103	"	5-7	-	4	221	50.68	107	33
-107	"	5-7	-	2	179	56.98	149	35
- 54	49=23 <sub>II</sub> +3 <sub>I</sub>	2-3	+	4	323	68.42	131	24
- 64	"	3-5	-	4	199	77.89	86	37
- 25	50=23 <sub>II</sub> +4 <sub>I</sub>	4-8	+	7	238	68.49	119	66
- 50	"	4-8	+	7	496	37.50	117	57
- 28	"	4-6	-	6	386	14.77	100	28
- 82	"	3-6		2	148	45.95	113	14
- 26	50=24 <sub>II</sub> +2 <sub>I</sub>	2-12	-	4	151	37.75	84	24
- 55	"	1-6	+	6	143	6.99	80	34
- 95	"	2-4	-	5	262	73.66	101	26
- 56	51=22 <sub>II</sub> +7 <sub>I</sub>	7-11	-	7	365	13.15	105	52
-117	"	7	-	5	348	63.79	142	19
- 2	51=23 <sub>II</sub> +5 <sub>I</sub>	4-7	+	3	316	70.57	102	40
- 13	"	5-7	+	5	292	45.21	119	39
- 33	"	5-10	-	3	294	69.73	133	48
- 58	"	5-7	+	3	337	85.16	125	27
- 74	"	5-7	-	4	123	73.86	129	39
-111	"	4-9	+	2	276	23.55	140	32
-116	"	5-7	+	2	159	92.45	129	28
- 46	51=24 <sub>II</sub> +3 <sub>I</sub>	3-5	+	4	330	24.85	136	44
- 19	"	1-5	+	3	256	47.66	149	36
- 60	51=25 <sub>II</sub> +1 <sub>I</sub>	1-5		5	95	23.16	83	26
- 34	52=22 <sub>II</sub> +8 <sub>I</sub>	6-10	+	3	219	38.36	107	13
- 35	52=23 <sub>II</sub> +6 <sub>I</sub>			3	355	42.82	137	51
- 89	"	6-8	+	2	160	35.63	125	30
- 41	52=24 <sub>II</sub> +4 <sub>I</sub>	4-6		4	253	67.98	135	51
- 76	"	4-6	-	6	315	77.14	107	22
- 78	"	3-4	-	3	219	79.45	134	52
-100	"	4-6	-	2	303	86.47	154	19
- 15	53=23 <sub>II</sub> +7 <sub>I</sub>	5-9	+	3	188	4.79	111	44
- 75	53=24 <sub>II</sub> +5 <sub>I</sub>	4-	+	3	490	40.20	162	27
- 81	"	4-5	+	3	371	40.43	129	26
- 85	54=25 <sub>II</sub> +4 <sub>I</sub>	4-8	+	3	149	33.56	117	19
- 88	53=25 <sub>II</sub> +3 <sub>I</sub>	3-5	-	2	145	90.34	132	24

Most of  $BF_2$  plants grew vigorously but the majority had more or less shorter culms than the parental species of which culms were 127 cm long on an average (NISHIYAMA 1929). Some segregates had much reduced height of culms, shorter than 95 cm, but they usually produced numerous culms. Such dwarf plants often had a much smaller number of spikelets per ear and usually exhibited high sterility. Besides, some  $BF_2$  were so weak that cytological material was unsatisfactory and in most cases they died before maturity.

$BF_3$  offspring were grown from a  $BF_2$ , 29-390-88, with  $53 = 25_{II} + 3_I$  and the data obtained so far are given in Table 8. Except in a few cases, the segregates were moderately fertile but had very short culms. It is clearly shown that two  $BF_3$  with  $25_{II}$  were of dwarf habit and bore seed in 47.65-66.28% by selfing. In the next generation their offspring were also found to be semi-fertile, 45.05% on an average.

Table 8. Fertility of  $BF_3$  (progenies from 29-390-88,  $25_{II}+3_I$ )

Plant No.	No. of chromosomes	Fluctuation of univalents	Chromosome complexes	No. of ears	No. of florets	% of seeds	Length of culms (cm)	No. of culms
30-467-1	$54=26_{II}+2_I$	1-4	+	7	421	80.29	104	63
-2	$50=25_{II}+0_I$	0-6	+	4	86	66.28	75	24
-5	$51=25_{II}+1_I$	1-?	-	4	125	70.40	74	31
-6	$52=25_{II}+2_I$	1-7	+	6	323	72.76	84	26
-7	$54=26_{II}+2_I$	2-6	-	6	263	60.46	92	24
-9	$53=25_{II}+3_I$	2-5	+	5	169	33.14	79	19
-10	$52=25_{II}+2_I$	2-4	+	9	244	12.70	72	32
-11	$51=25_{II}+1_I$	1-3	-	3	179	80.45	106	16
-12	$52=25_{II}+2_I$	2-4	-	3	199	74.37	115	34
-14	$50=25_{II}+0_I$	0-4	-	7	170	47.65	68	22

Table 9 represents the results of the same observation on the progenies from a  $BF_2$ , 29-390-95, with  $24_{II} + 2_I$ . All segregates having 47 and 49 chromosomes showed a good production of seeds. But the offspring with 48 chromosomes,  $24_{II}$ , were low fertile and had very short culms.

Table 9. Fertility of  $BF_3$  (progenies from 29-390-95,  $24_{II}+2_I$ )

Plant No.	No. of chromosomes	Fluctuation of univalents	Chromosome complexes	No. of ears	No. of florets	% of seeds	Length of culms (cm)	No. of culms
30-456-5	$47=23_{II}+1_I$	1-	-	2	157	90.45	89	38
-8	"	1-3	-	4	196	82.14	70	40
-13	"	0-1	+	2	122	91.80	86	27
-7	$48=24_{II}+0_I$	0-4	-	3	116	14.66	66	41
-17	"	(0-4)?	-	2	70	15.71	53	37
-2	$49=24_{II}+1_I$	1	-	3	221	90.05	90	34
-6	"	1-3	-	3	160	97.50	79	26
-9	"	1-3	-	2	188	94.15	106	25

Hyperhexaploids with  $22_{II}$  which were derived from a  $BF_2$  possessing 46 chromosomes showed no remarkable difference in the fertility and vigour from sister plants differing in chromosome number (Table 10). However, in comparison with pure species, they are somewhat inferior in seed production and vegetable development.

Table 10. Fertility of  $BF_2$  (progenies from 29-390-11,  $22_{II}+2_I$ )

Plant No.	No. of chromosomes	Fluctuation of univalents	Chromosome complexes	No. of ears	No. of florets	% of seeds	Length of culms (cm)	No. of culms
30-440-10	$43=21_{II}+1_I$	0-3	+	2	202	68.81	85	28
-19	"	0-3	+	2	207	69.57	90	22
-1	$44=22_{II}+0_I$	0-2	+	4	538	74.16	86	23
-11	"	0-4	—	1	122	79.51	78	13
-13	"	0-6	—	3	465	81.29	84	37
-14	"	0-4	+	2	244	78.28	86	26
-12	$45=22_{II}+1_I$	1-3	—	3	342	82.16	82	31
-18	$46=22_{II}+2_I$	1-	+	2	227	64.76	90	27

Table 11. Fertility of  $BF_2$  (progenies from 29 370-1,  $49=20_{II}+9_I$ )

Plant No.	No. of chromosomes	No. of ears	No. of florets	No. of seeds	% of seeds
32-517-2	$42=20_{II}+2_I$	2	237	18	7.59
-9	$42=20_{II}+2_I$	3	327	88	26.91
-8	$42=21_{II}+0_I$	3	236	190	80.51
-5	$43=21_{II}+1_I$	3	277	274	98.92
-1	$44=21_{II}+2_I$	2	309	289	93.53
-4	$44=21_{II}+2_I$	3	155	126	81.29

Table 11 gives further data on the seed production of  $BF_2$  with 42-44 chromosomes. Two plants with  $42=20_{II}+2_I$  exhibit high sterility. While the others, having  $21_{II}$  or  $21_{II}+(1-2)_I$ , are fertile up to 81-99%.

From the results given above it may be concluded that anomalous numbers or combinations of chromosomes are no doubt the principal cause which induces sterility and also weakness of plant development. But within limits between certain chromosome numbers it is difficult to find a definite correlation between sterility and chromosome numbers. The constantly high production of seeds is observed only in plant strains which have 21 and 22 bivalents, while hyperhexaploids with  $24_{II}$  and  $25_{II}$  are very low or semi-fertile. It should also be mentioned that all the hyperhexaploids described just above have a semi-dwarf habit.

### Summary

(1) Pentaploid hybrids between tetraploid and hexaploid *Avena* species are completely or highly sterile. This sterility is

clearly due to the gametic abortion which is caused by the abnormal maturation divisions.

(2) The cytological observation was extensively made on selfed ( $F_2$ - $F_4$ ) and back-crossed progenies ( $BF_1$ - $BF_5$ ). Their chromosome number changes in a very wide range,  $2n = 42-67$ . Also the chromosome configuration is usually quite variable in the same plant, and varies even more from plant to plant, but there were obtained hyperhexaploids possessing  $22_{II}$ ,  $24_{II}$  and  $25_{II}$  in the back-crossed offspring and also an octoploid plant with  $28_{II}$  in the  $F_4$  progeny. The offspring with  $22_{II}$  bred true, but the others did not, because some chromosomes occasionally or often failed to pair at meiosis. Besides, the author got two constant types, one is the euhexaploid plant ( $n = 21$ ), and the remaining is the unexpected diploid plant ( $n = 7$ ) as reported in his previous paper (NISHIYAMA 1933).

(3) Hybrid progenies showed a wide variation in their fertility and plant-vigour. It is especially mentioned that hexaploid derivatives with  $21_{II}$  and hyperhexaploids with  $22_{II}$  are highly fertile, but those with  $24_{II}$  and  $25_{II}$  are low or semi-sterile. All of these hyperhexaploids show the semi-dwarf habit.

In conclusion, I have great pleasure in recording my gratitude to Professor H. KIHARA for much helpful criticism and guidance.

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### Cytogenetical Studies in *Avena*. III

#### Experimentally produced eu- and hyperhexaploid aberrants in oats \*

By

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As stated in the preceding paper, pentaploid *Avena* hybrids were karyologically unbalanced and gave rise to alternations of chromosome combination in the offspring. Out of many segregates with these new chromosome combinations, three constant strains were established in later selfed and back crossed progenies of the hybrid between *Avena fatua* ( $n=21$ ) and *A. barbata* ( $n=14$ ). Two strains showed 21 pairs of chromosomes at meiosis and the remaining one 22 pairs (NISHIYAMA 1939).

In the present account is given chiefly a further study on the qualitative analysis of the chromosome complement in these constant forms.

In external morphology these new forms approached *A. fatua*, but showed many minor distinctions from it and among themselves. Before describing the cytological observation, therefore, their morphological characteristics will be briefly indicated.

Morphology of *A. fatua*: Culms semi-erect in early growth period, 127 cm long, ears equilateral, wide spreading, drooping, 80 spikelets, basilar articulation distinct, all florets readily separating from their axes in ripening, basal hairs present in bushy ring, empty glume ca. 22 mm long, lemma dark brown, covered with long hairs, awns twisted and geniculated (cited from NISHIYAMA 1929).

In the three strains, principal characters which are distinct from *A. fatua* are as follows:

Pc-1 (BF<sub>2</sub> with 21 bivalents in Table 4, NISHIYAMA 1939); culms nearly normal height, 110 cm, lemma (or grain) somewhat longer, brown, ca. 74 spikelets per panicle.

Pc-2 (BF<sub>4</sub> with 21 bivalents in Table 4, NISHIYAMA 1939); Semi-dwarf, i.e. culms ca. 75 cm long, grain and awns somewhat longer, lemma brown, empty glume longer but narrower, ca. 36 spikelets per panicle.

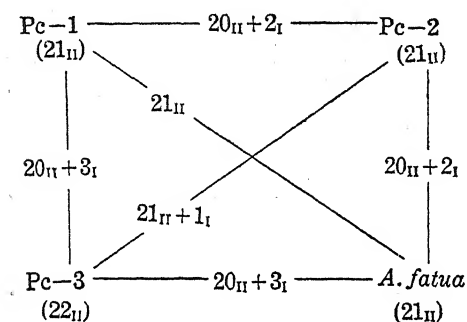
Pc-3 (BF<sub>4</sub> with 22 bivalents in Table 4, NISHIYAMA 1939); Semi-dwarf, i.e. culms 70 cm long, grain somewhat smaller, blackish

\* Contributions from the Laboratory of Genetics, Biological Institute, Kyoto Imperial University No. 104.

brown like *A. barbata*, lemma of the first grain carries a small number of short hairs, the second grain usually glabrous, empty glume longer but narrower, ca. 40 spikelets per panicle.

The fertility of these new forms is usually normal, but is often variable, probably being influenced by environmental condition at the time of flowering.

Among the three strains and *A. fatua*, all of six possible crosses were made and  $F_1$  hybrids were used for the study on the meiotic pairing of chromosomes. The cytological results are summarized in the following scheme. That is, it means that the hybrid between Pc-1 ( $n = 21$ ) and *A. fatua* ( $n = 21$ ) shows twenty-one chromosomes closely paired at first metaphase in PMC, and so on (Fig. 1a).



Scheme showing chromosome affinity in the hybrids among four oat strains.

In Pc-2 ( $n = 21$ )  $\times$  *A. fatua* ( $n = 21$ ) twenty bivalent chromosomes are formed in a normal manner but the remaining two show no tendency toward pairing. Similar chromosome pairing is also found in Pc-3 ( $n = 22$ )  $\times$  *A. fatua* ( $n = 21$ ) but in this case one extra chromosome of the former is also left unpaired. Accordingly, this hybrid always

exhibits  $20_n + 3_i$  at first metaphase (Fig. 1c). In Pc-3 ( $n = 22$ )  $\times$  Pc-2 ( $n = 21$ ), the chromosome configuration is usually  $21_n + 1_i$  and in rare cases  $20_n + 3_i$ . From these facts it probably may be said that both of these strains have a similar chromosome complement, with the exception of one extra chromosome of Pc-3. The chromosome pairing  $20_n + 2_i$ , in the hybrid, Pc-1 ( $n = 21$ )  $\times$  Pc-2 ( $n = 21$ ), and  $20_n + 3_i$  in Pc-1 ( $n = 21$ )  $\times$  Pc-3 ( $n = 22$ ) may readily be understood from the facts mentioned just above (Fig. 1b).

Judging from their size and shape, showing a median or sub-median constriction, two univalents from the hybrids with  $20_n + 3_i$  appear always to correspond with those of the hybrids with  $20_n + 2_i$ . The third univalent of the former has also a median constriction, but is the smallest. From these facts it seems probable that in these hybrids certain specific chromosomes always fail to pair.

These cytological evidences suggest strongly that Pc-2 has a reconstruction complement of chromosomes, containing twenty pairs of *fatua* chromosomes and one pair of *barbata* chromosomes which is unable to conjugate with any of the *fatua* chromosomes. Pc-3



also possesses the same complement as Pc-2 and one pair of specific *barbata* chromosome in excess. In the last strain, Pc-1, the chromosome situation appears to be completely homologous with that of *A. fatua*.

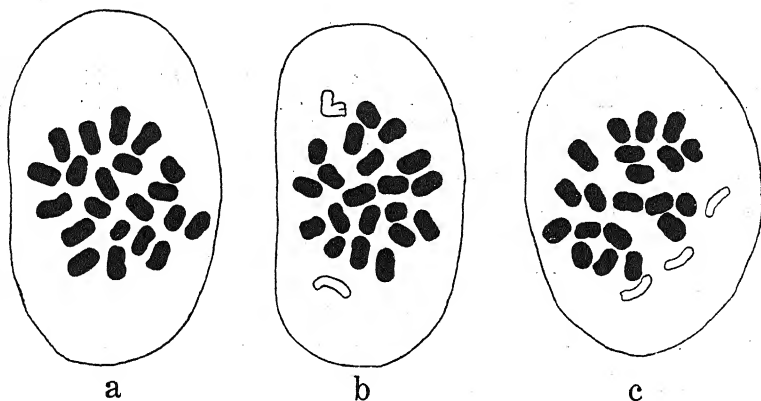


Fig. 1. Chromosomes at first metaphase in PMC. a, Pc-1  $\times$  *A. fatua*,  $21\text{II}$ ; b, Pc-1  $\times$  Pc-2,  $20\text{II}+2\text{I}$ ; c, Pc-3  $\times$  *A. fatua*,  $20\text{II}+3\text{I}$ .

The genom formula of *A. fatua* and *A. barbata* was shown by the symbol AAB $\overline{\text{B}}$ CC and AAB'B' respectively (NISHIYAMA 1934, 1939). If we assume that the B genom contains  $b_1b_2b_3b_4b_5b_6b_7$  chromosomes, *A. fatua* and Pc-1 might be represented by the formula AACC +  $2 \times b_1b_2b_3b_4b_5b_6b_7$ . While Pc-2 would be shown by AACC +  $2 \times b_2b_3b_4b_5b_6b_7 + 2\beta_1$  and Pc-3 by AACC +  $2 \times b_2b_3b_4b_5b_6b_7 + 2\beta_1 + 2\beta_2$  in which  $\beta_1$  and  $\beta_2$  are specific *barbata* chromosomes.

Although these new forms markedly resemble *A. fatua*, in some external characters they are like *A. barbata* or distinct from either of the parental species. The origin of these new or *barbata*-like characters would probably be assumed to be partly due to the reconstruction of the chromosome complement referred to above and partly due to the replacement of some *fatua* chromosomes by *barbata* homologues (cf. NISHIYAMA 1934). According to NISHIYAMA (1929) it has already been found that the maximum number of paired chromosomes amounted to 11 in the hybrid between *A. barbata* and *A. fatua*. This cytological fact might support the possible occurrence of such a chromosomal exchange between the two species without any cytological disturbance.

There have been reported some similar cases in which new hyperpolyploid forms are experimentally produced. COLLINS, HOLLINGSHEAD and AVERY (1929) found a new species *Crepis articalis* ( $n = 12$ ) from the interspecific hybrid *C. biennis* ( $n = 20$ )  $\times$  *C. setosa* ( $n = 4$ ). Its chromosome complement consisted of ten pairs

from *biennis* and two pairs of *setosa* chromosomes. WEBBER (1930) obtained a distinct new type with twenty-five bivalents from the offspring of the sesquidiploid *Nicotiana* hybrid which probably had arisen from the union of a diploid *tabacum* egg and a haploid *sylvestris* sperm. Later LAMMERTS (1932) also established a new strains having  $n = 24$  *rustica* + 6 *paniculata* chromosomes by selfing the back cross hybrid [*Nicotiana rustica* ( $n = 24$ )  $\times$  *paniculata* ( $n = 12$ )]  $\times$  *rustica* ( $n = 24$ ). Finally it was shown by UCHIKAWA (1937) that the short *compactum* type contained twenty-two bivalents from which one bivalent was reduplicated in excess as compared with normal *vulgare* wheat ( $n = 21$ ).

A similar chromosome situation as in Pc-2 has already been found by KIHARA and WAKAKUWA (1935) in giant types which arose spontaneously from dwarf wheat with twenty bivalents. These giant wheats were clearly euhexaploid as usual but had nineteen different and two duplicated pairs of chromosomes.

Since these aberrants have new chromosome complements as well as new and different plant characters they might be considered as new varieties or might require more distinctive taxonomic recognition.

In short, the present work provides a further example of the induction of aberrants with quantitatively and qualitatively altered complements of chromosomes which are due to the recombination of chromosomes from two distinct plant species.

In conclusion I wish to acknowledge my gratitude to Professor H. KIHARA for much advice and criticism.

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## Studies on the Protoplasmic Nature of Stimulation and Anesthesia<sup>1)</sup>

By

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In the first part of this paper experiments are reported which indicate that stimulation of *Spirogyra* protoplasm by uneven pressure and hypertonic sucrose solutions cause a decrease in the elasticity (structural viscosity) of the protoplasm. In the second part experiments are recorded which indicate that initially anesthetics cause a decrease in structural viscosity but with longer immersions in anesthetics the structural viscosity returns to approximately normal. However, the experiments will demonstrate that although the structural viscosity returns to approximately normal, the structure of the protoplasm is not normal because the protoplasm cannot be stimulated by an electrical current as can normal protoplasm; that is, the protoplasm is anesthetized.

### Stimulation

Before the effects of stimulants on protoplasm can be discussed it is essential to know the structure of protoplasm in unstimulated cells. Northen (1938a) and Northen and Northen (1938) have demonstrated that protoplasm in cells of *Spirogyra* is an elastic fluid (one which possesses structural viscosity). From a biochemical point of view that would be expected because, except at high temperatures, many protein solutions possess structural viscosity (Schmidt, 1938). The concept of protoplasm which best fits our investigations on the structure of protoplasm is that suggested by the biochemist Block (Schmidt, 1938). Block states that it appears that polypeptides as they are present in actively metabolizing centers, may be organized in a loosely knit grid formation including in the grid not only polypeptides but possibly also lipoids and other cell constituents. This concept of protoplasm as a network, a pattern of proteins and lipoids with other cell constituents has proved useful in explaining the effect that heat (Northen, 1939) and cations (Northen and Northen, 1939) have upon protoplasm.

Block (Schmidt, 1938) suggests the protoplasmic nature of

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1) Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 176.

stimulation. He states that the complex grids can be easily changed by the organism or by physical and chemical manipulations into the globular proteins composed of reversibly dissociable systems. In other words, in unstimulated protoplasm the proteins are mostly in the form of long chains and these chains are associated with lipoids and other cell constituents. Upon stimulation some of the proteins are separated from the grid and the proteins change from a long chain form into a globular form. The separation of the proteins from the grid and their change to a globular form would result in a decrease in structural viscosity. All of the stimulating agents which we have studied do cause a decrease in the structural viscosity (elasticity) of the protoplasm of *Spirogyra*. The list includes injury (Northen and Northen, 1938), anesthetics (Northen, 1938b), heat (Northen, 1939), sound (Northen and MacVicar, 1939), electricity (Northen and MacVicar, 1939), and X irradiation (MacVicar, investigation in progress). In this paper it will be demonstrated that uneven pressure and hypertonic solutions also cause a decrease in the structural viscosity of *Spirogyra* protoplasm. Hence irrespective of the diverse nature of the stimulating agents the initial protoplasmic reaction is apparently the same. The reaction results in a decrease in the structural viscosity of the protoplasm.

### Experimental

In this part data will be presented which indicate that hypertonic sucrose solutions and uneven pressure cause a decrease in the structural viscosity of protoplasm in cells of *Spirogyra*.

**Osmotic stimulation.** Filaments of *Spirogyra* were immersed for various periods of time in different concentrations of sucrose which were made up in tap water. The filaments were then centrifuged with an acceleration of  $382.5 \times$  gravity for two minutes. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced in experimental and control groups were determined. About 300 filaments were used for each group. The data are summarized in table 1.

Table 1. Effect of hypertonic solutions on the structural viscosity of protoplasm

Minutes immersed	Percentages of filaments in the cells of which the chloroplasts were displaced when immersed in			
	Water	.2 molal sucrose	.3 molal sucrose	.4 molal sucrose
10	18%	90%	97%	—
20	28	67	94	—
25	32	64	93	93%
40	23	54	88	—

The filaments which had been immersed in .2 molal sucrose were slightly plasmolyzed. In the other concentrations plasmolysis was more pronounced. It will be noted, table 1, that in all instances the chloroplasts were displaced by the centrifugal acceleration in more filaments which had been immersed in sucrose solution than in those which remained in water. The chloroplasts were not displaced in most control filaments because protoplasm has a yield value and the force resulting from the centrifugal acceleration was less than the yield value. Northen (1938a) has demonstrated that the velocity with which the chloroplasts move in response to a centrifugal acceleration can be approximately calculated from the equation,  $V = k(c - c_0)$ , in which  $V$  is the velocity of chloroplastic movement,  $k$  is a constant,  $c$  is the centrifugal acceleration used, and  $c_0$  is the initial starting acceleration (the yield acceleration). For most control filaments  $c_0$  was equal to or greater than  $382.5 \times$  gravity. Hence when such filaments were centrifuged with an acceleration of  $382.5 \times$  gravity the velocity would be zero because  $V = k(382.5 - 382.5) = 0$  or  $V = k(382.5 - > 382.5) = < 0$ . Because protoplasm has a yield value it is an elastic fluid (one with structural viscosity) and it is likely that the elasticity results from a grid structure such as Block postulated.

The chloroplasts moved in most filaments which were immersed in the hypertonic solutions because the contraction of the protoplasm loosened the grid structure and concomitantly lowered the value of  $c_0$  to a value less than  $382.5 \times$  gravity. Hence in the stimulated filaments,  $V = k(382.5 - < 382.5) = > 0$ .

The experiment furnishes additional support to the concept that protoplasm has a net-like ultramicroscopic structure which can be readily loosened by stimulating agents. It is highly probable that the chloroplasts moved in more stimulated filaments than in unstimulated filaments because of a structural change, that is the separation of the proteins from other constituents and the change in shape of the protein molecules, which would loosen the structure and accordingly lower the value of  $c_0$ . Unquestionably it was not a decrease in *true* viscosity which enabled the centrifugal acceleration to displace the chloroplasts in the stimulated filaments because in colloidal suspensions which have true viscosity a decrease in water content does not decrease the viscosity but increases it.

**Uneven pressure.** Filaments of *Spirogyra* were placed in water on microscope slides. Control groups were left uncovered whereas experimental groups were covered with a cover glass (weight = .16 g.). Different weights were then placed on the cover glasses. After the weights had acted the desired time the filaments were

centrifuged with an acceleration of  $680 \times$  gravity for one minute. About two hundred filaments were used for each group. The data are summarized in table 2.

Table 2. Effect of uneven pressure on structural viscosity

Minutes weight acted	Percentages of filaments in the cells of which the chloroplasts were displaced when covered with the following weights						
	0 grams	2 grams	5 grams	10 grams	20 grams	50 grams	100 grams
5*	13%	41%	50%	52%	36%	47%	61%
2	1	1	2	—	18	—	—
4	2	28	22	—	34	46	68
6	1	13	5	—	42	—	—
8	3	3	7	—	32	—	—
15	4	32	43	—	32	—	—

\* This series was performed on October 15. Subsequent experiments were performed on October 16.

The data in table 2 indicate that uneven pressure decreases the structural viscosity of the protoplasm probably through loosening the protoplasmic grid. When two gram and five gram weights were used the structural viscosity initially decreased, then increased to about normal, and then decreased again.

### Anesthesia

Northern (1938) has found that when filaments of *Spirogyra* are immersed in fat solvent anesthetics the structural viscosity of the protoplasm decreases for the first few minutes but after longer immersion returns to normal or may in some instances becomes slightly greater than normal. However in the latter instance the protoplasm is not coagulated because the chloroplasts may be moved with a slightly higher centrifugal acceleration than that required for normal cells. However, the return to approximately normal structure does not mean a return to the original protoplasmic pattern. The reconstructed pattern differs from the normal pattern for though still immersed in the anesthetic the filaments no longer responded as did the protoplasm initially. The reconstructed pattern is not as sensitive to stimulation as was the normal pattern. In other words, the protoplasm is anesthetized. In the experiments which will be recorded it will be demonstrated that the reconstructed pattern cannot be stimulated by electricity whereas normal protoplasm can be stimulated by the electrical current.

### Experimental

Filaments of *Spirogyra* were immersed for various periods of time (see tables 3 and 4) in 4% ether. After they had been im-

mersed for the desired time one lot was centrifuged in ether with an acceleration of  $382.5 \times$  gravity for two minutes and another lot was transferred to water and subjected to a direct current of 2 ma. for 2 minutes according to the method previously described by Northen and MacVicar (1939). Such filaments were then centrifuged with an acceleration of  $382.5 \times$  gravity for two minutes. Control filaments were also centrifuged with an acceleration of  $382.5 \times$  gravity for two minutes. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced (moved to the centrifugal end) were determined for experimental and control groups. In those filaments which were subjected to the electrical current the region near the anode was selected for study. About 200 filaments were used for each group. Five experiments were performed. Essentially they were all in agreement. The data for experiment 1 are summarized in table 3.

Table 3. Effects of ether on stimulation

Minutes in ether	Percentage of filaments in the cells of which the chloroplasts were displaced when treated with			
	Ether only	Ether followed by electricity	Water only	Electricity only
0	—	—	10%	59%
2	87%	83%		
5	60	73		
10	9	52		
15	31	27		
20	11	13		

In experiment 1 it will be noted that after immersions of two and five minutes the chloroplasts were displaced in the cells of more filaments exposed to ether than in the water control. This indicates that initially ether decreases the structural viscosity of the protoplasm. The decrease in structural viscosity probably results from a separation of the proteins from the protoplasmic grid and their concomitant change from chain to a globular form. Once the normal pattern, the grid, is broken down the opportunity exists for the grid constituents to assume new combinations and probably shapes. With respect to the proteins Block (Schmidt, 1938) states that the soluble globular proteins may in turn be converted into microcrystalline fibrous substances having a lower degree of solubility. The new combinations and probably forms cause the structural viscosity to again increase so that at the end of twenty minutes immersion in ether the structural viscosity is approximately normal. However the new pattern, the new combination, differs from the normal pattern. The new pattern is a relatively insensitive one—it is an

anesthetized one—because it cannot be stimulated by electricity to the degree that the normal pattern can. It will be noted that in those filaments which were exposed to ether for twenty minutes before being subjected to electricity the chloroplasts were displaced by the centrifugal acceleration in cells of only 13% of the filaments whereas in the filaments which were not exposed to ether prior to electricity the chloroplasts were displaced in cells of 59% of the filaments. The data for experiment 2 are recorded in table 4.

Table 4. Effects of ether on stimulation

Minutes in ether	Percentage of filaments in the cells of which the chloroplasts were displaced when treated with			
	Ether only	Ether followed by electricity	Water only	Electricity only
0	—	—	16%	72%
5	55%	72%		
10	37	74		
20	4	50		
40	20	24		

The fact that after 40 minutes immersion in ether the chloroplasts were displaced in 20% of the filaments and were displaced in 16% of the water control filaments suggests that the structural viscosity of the anesthetized and non-anesthetized protoplasm was approximately the same. That such is true was further evidenced by centrifuging a group of control filaments and a group of filaments which had been immersed in ether for 40 minutes with an acceleration of  $1062 \times$  gravity instead of with an acceleration of  $382.5 \times$  gravity. Following a centrifugation of  $1062 \times$  gravity for 30 seconds the chloroplasts were displaced in 43% of the anesthetized filaments and in 48% of the non-anesthetized filaments thus indicating that the structural viscosity in the two groups was approximately the same. Although the structural viscosity was approximately the same the protoplasmic pattern in the two groups was not the same because the anesthetized protoplasm could not be stimulated by the electrical current whereas the non-anesthetized protoplasm could be stimulated.

The comparatively unsensitive pattern of the anesthetized protoplasm may, however, be restored to the normal sensitive pattern. At the time experiment 2 was performed a group of filaments were immersed in ether for 40 minutes and then transferred to water where they were allowed to remain for 90 minutes. They were next exposed to a current of 2 ma. for 2 minutes and were then centrifuged with an acceleration of  $382.5 \times$  gravity for 2 minutes. Another group was immersed in ether for 40 minutes, exposed to a current



of 2 ma. for 2 minutes, transferred to water and allowed to remain there for 90 minutes, and then were given a current of 2 ma. for 2 minutes. Finally they were likewise centrifuged with an acceleration of  $382.5 \times$  gravity for 2 minutes. The results are tabulated below.

Treatment	Percentages of filaments in the cells of which the chloroplasts were displaced
Water (control)	16%
Electricity (control)	72%
Ether 40 min.; elect. 2 min.	24%
Ether 40 min.; water 90 min.; elect. 2 min.	87%
Ether 40 min.; elect. 2 min.; water 90 min.; elect. 2 min.	84%

The results of another experiment which was performed at a different time are tabulated below. The filaments were centrifuged with an acceleration of  $382.5 \times$  gravity for 2 minutes.

Treatment	Percentages of filaments in the cells of which the chloroplasts were displaced
Water (control)	11%
Electricity 2 min. (control)	62%
Ether 60 min.; elect. 2 min.	30%
Ether 60 min.; water 60 min.; elect. 2 min.	77%
Ether 60 min.; elect. 2 min.; water 60 min.; elect. 2 min.	60%

The data indicate that the protoplasm can come out of the state of anesthesia. It will be noted that after the filaments had been in water for 90 minutes or 60 minutes the protoplasm was again sensitive to the electrical current.

### Summary

Uneven pressure and hypertonic sucrose solutions decrease the structural viscosity of protoplasm in cells of *Spirogyra* as evidenced by the fact that an acceleration which would not displace the chloroplasts in most cells of control filaments would displace the chloroplasts in most cells of filaments which had been stimulated by uneven pressure or hypertonic sucrose solutions. Presumably the decrease in structural viscosity results from the separation of the proteins from the protoplasmic network and their concomitant change from a chain to a globular form.

Following short immersions in ether the structural viscosity of *Spirogyra* protoplasm, as determined by the centrifuge method, is less than that of normal protoplasm. After longer immersions in ether the structural viscosity returns to approximately normal.

Although the structural viscosity returns to approximately normal the protoplasmic pattern does not, because the altered protoplasmic pattern cannot be stimulated by an electrical current as can the normal protoplasmic pattern; that is, after long immersions in ether the protoplasm is anesthetized as evidenced by the observations that in normal cells an electrical current of 2 ma. acting for 2 minutes causes a decrease in structural viscosity whereas in anesthetized cells the electrical current does not cause an appreciable decrease in the structural viscosity. Biochemically the course of anesthesia may be as follows: the initial break down of the protoplasmic network (as evidenced by the decrease in structural viscosity) is followed by a rearrangement of the network constituents. The rearranged pattern is a relatively non-sensitive one. However, the original sensitive pattern can be obtained again by allowing the anesthetized filaments to remain in water for 60 or more minutes following the ether-treatment.

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## Regeneration of Gonads in *Plecoglossus altivelis* after Spawning Season

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### Introduction

*Plecoglossus altivelis* (Ayu in Japanese) is very common in Japan, from the southern part of Hokkaidō to Korea and Formosa. The fish hatched during the previous autumn begin to spawn in this autumn. As soon as the temperature of the water falls to below 2°C, the fish begin to descend the streams with the purpose of performing the spawning, and seek the spawning ground. It is generally known that the fish die soon after they have finished their spawning. In fact the dead fish are observed in the ground of the rivers from the middle of October to January. On account of the above stated fact, the fish is called "Nengyo" meaning that the life cycle is completed within one year.

One of the most interesting facts is, however, that some fish are able to pass the spawning season successfully. Such fish are called "Otunen-Ayu" or "Huruse" in Japanese. According to the above description, we have from the oecological point of view two types in the life history of the fish. One type of the fish is observed in the spawning season, i.e., from the latter part of September until the middle of December (A period), while the other is observed after the spawning season, i.e., from January until June (B period).

My honoured teacher Hon. Prof. Chiyomatsu Ishikawa has been much interested in such Ayu passing over the spawning season, so he visited various rivers, Lake Biwako, Lake Ikedako, Unagiike Pond, etc. to study the life cycle of this fish. And then, he began to study the cause of the fish's surviving the spawning season by means of a microscopical examination of the gonads.

In consequence of Hon. Prof. Ishikawa's death\*, the investigation was continued by the writer at the Botanical Institute, College of Agriculture, Tokyo Imperial University, following his idea. This paper contains the results of the research of seasonal change of the fish and the microscopical examination of their gonads.

The writer wishes to express his most cordial thanks to late Dr. C. Ishikawa, Hon. Prof. of the Tokyo Imperial University, for his kind direction, and to Prof. T. Ogata of the medical department of the Tokyo Imperial University for his valuable suggestion and for reading the manuscript. Thanks are also due to Dr. Sanae Takata, ex-president of the Waseda University, Prof. K. Miyake, and Mr. K. Ishikawa, the son of late Dr. Ishikawa, who aided him in various ways.

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\*He attended the 10th meeting of Japanese Association for the Advancement of Science held at Taihoku, Formosa in December, 1934. There he passed away at the age of 75 in the morning of January 17th.

## Materials and Methods

The material used in this investigation has been collected from the following localities (table 1). The river Tamagawa near the Tokyo City, the river Kumagawa in the Kumamoto Prefecture, Unagiike Pond in the Kagosima Prefecture, Yosino Experiment Station on Fisheries of the Tokyo Prefecture Office, the fish firm of Samezima near the Tokyo City, the river Tansui near the Taihoku City. And the material which Dr. Ishikawa collected at Unagiike Pond was also used.

The fish of Unagiike Pond are small, about 8 cm. in length. One of the most interesting facts on Ayu fish is that there is a land-locked form in Lake Biwako known as "Koayu", i.e. "small Ayu". In his short paper on the fishes of Lake Biwako Ishikawa (1895) reported that the "Koayu" appears to be identical with *P. altivelis*, but it does not seem to attain the size of the latter, the individuals with ripe eggs remaining only in the length of 7. or 8. cm. He was not sure whether it was a form different from the ordinary Ayu or not at the time, so he has tried to find out the cause of the smallness of this Ayu by cultivation in pond or in transferring them into rivers. As a result he found that the smaller forms are of the same species simply dwarfed by land-locking.

If his view of the origin of small Ayu be valid, we must be able to produce them in any place just in the same way as small Ayu is produced in Lake Biwako. With this idea he began to look after the lakes and ponds in the different parts in Japan. He found Lake Ikedako and Unagiike Pond being exactly what he thought of. Ayu fish were not found before in Lake Ikedako and Unagiike Pond, and the fish in these places are progeny of those produced by subsequent transportation of the ordinary fish from other place.

Collections of the living fish used in this study were made from the middle of September until the middle of June. The gonads of the living fishes were fixed with Bouin's fluid. The sections were 7-12 micra thick and stained with Heidenhain's iron-alum-haematoxylin.

Here, the writer wishes to express his gratitude to Prof. N. Takahashi of the Fifth Higher School, Kumamoto and Prof. K. Hirasaka of the Taihoku Imperial University, and Mr. S. Torii, for supplying him with the fish used for the present study. The writer also wishes to acknowledge his indebtedness to Yosino Experiment Station on Fisheries of the Tokyo Prefecture Office, for abundant material at his disposal and facilities offered during this work.

Table 1

Time of collection	Locality	Body length (cm.)	Number	Sex
Sept. 25, 1935	Unagiike Pond	7-8.50 7-9.00	15 13	♂ ♀
Sept. 30, 1935	Yosino Experiment Station on Fisheries	14-19.00 14-20.00	12 10	♂ ♀
Oct. 18, 1934	The Tamagawa	14-22.00 15-21.00	6 2	♂ ♀
Oct. 22, 1934	The Tamagawa	12-20.00 14-16.00	5 2	♂ ♀
Oct. 14, 1935	Yosino Experiment Station on Fisheries	15-19.00 16-20.00	14 8	♂ ♀
Oct. 20, 1935	The Tamagawa	14-20.00 16-19.00	13 20	♂ ♀
Oct. 28, 1935	Yosino Experiment Station on Fisheries	15-18.00 15-20.00	16 13	♂ ♀
Oct. 15, 1935	Unagiike Pond	7-8.00 7-8.50	11 18	♂ ♀
Oct. 20, 1935	The Kumagawa	13-16.00	3	♂
Oct. 30, 1935	Unagiike Pond	7-8.00 7-8.00	10 13	♂ ♀
Nov. 11, 1935	Unagiike Pond	7-8.00 7-8.00	16 18	♂ ♀
Dec. 8, 1935	The Tamagawa	15.00 17-18.00	1 2	♂ ♀
Dec. 10, 1934	The Kumagawa	14-16.00	3	♂
Dec. 11, 1935	The Kumagawa	15-20.00 17-20.00	1 36	♂ ♀
Jan. 19, 1933	Unagiike Pond	8-8.50 7-8.00	18 23	♂ ♀
Jan. 19, 1935	The Tansui	19-24.00 20	4 1	♂ ♀
Jan. 22, 1935	The Tansui	20-21.00	3	♂
Jan. 19, 1936	The Tamagawa	17-20.00	11	♀
Jan. 31, 1936	Fish firm Samezima	17-20.00 18-20.00	10 5	♂ ♀
Feb. 7, 1936	The Tamagawa	18.00	1	♀
Feb. 9, 1936	The Tamagawa	14-20.00	13	♀
Feb. 15, 1936	Unagiike Pond	9-10.00 10-11.00	5 8	♂ ♀
Feb. 18, 1936	The Tamagawa	13-20.00	12	♀
Mar. 19, 1935	The Tamagawa	14-18.00	6	♀
Mar. 20, 1935	Unagiike Pond	12.00 13.00	2 2	♂ ♀
Mar. 17, 1936	Unagiike Pond	9-11.00 9-11.00	6 9	♂ ♀
Mar. 26, 1936	Unagiike Pond	10-12.00 10-11.00	5 5	♂ ♀
Apr. 21, 1935	The Tamagawa	15-17.00	5	♀
Apr. 26, 1935	The Tansui	19-21.00	3	♀
May 21, 1935	The Tamagawa	14-18.00	2	♀
May 15, 1936	Unagiike Pond	12.00 9-11.00	1 3	♂ ♀
May 7, 1935	The Tansui	13-22.00	4	♀
May —, 1936	Unagiike Pond	10-11.00 10-12.00	15 20	♂ ♀
June 18, 1935	The Tamagawa	16-20.00	6	♀

## Observations

In the A period the external features of the fish change in color and form. The change of the male fish is most remarkable and attracts our attention. The Ayu fish are famous for their beautiful form and colors among the fishes in our country, and in the ordinary season the color of the body does not vary with the sex. The head and dorso-lateral portion of the body is olive greenish brown; silvery white with luster on the side; the ventral portion is white or silvery white with yellow green; fins are greenish yellow. When the spawning season approaches, the change of the color of the body surface begins to appear gradually. The head and dorso-lateral portion of the body is steel black; the ventro-lateral portion reddish orange; the adipose fins and other fins are reddish orange.

During the spawning season, the occurrence of the pearl organs in various fishes are observed by the other investigators and its significance has been discussed. Of the present species the structure of the pearl organs is studied by K. Ebina ('29 '30). The pearl organs appear almost on the entire body surface, vis., head, gill cover, scales, adipose fin, and the rays of all the other fins. The organs do not occur in the female fish more than in the male fish. Finally the nuptial coloration and pearl organs thus appearing develop to a high degree in November and they are kept through all the spawning season.

When the spawning season is finished, i.e. in the B period, the nuptial coloration and the pearl organs begin to disappear gradually.

In the female fish, the head and dorso-lateral portion of the body is olivish gray; olive greenish gray on the side, the ventro-lateral portion is silver white, fins are yellowish brown. In the male fish, the head and dorso-lateral portion of the body is dark brownish gray, silver white without luster on the side, fins are faint orange. The male fish are emaciated without luster on the body surface. However, the female fish are of a very beautiful color like the spring form, perhaps a little darker.

## Female gonads

At the beginning of the breeding season, numerous fish have many opaque immature eggs in the ovaries (fig. 3), and the body cavity of some fish is full of ripe eggs with yellow color (fig. 2), so that they come out on squeezing. At the middle of the breeding season, the body cavity of a large majority of the fishes is full of well grown eggs. However, some of fish have already detached the eggs, and only a few ripe eggs are observed in the ovaries. At the end of breeding season, numerous fish have already laid the eggs, bearing a few ripe eggs in the ovaries. However, some fish have not yet detached them, and their body cavity is full of well developed eggs.

It is one of the most interesting facts that the specimens of Unagiike Pond show a particular development of the ovaries on the middle of the season. In these specimens, the right ovaries are filled with large and ripe eggs with yellow color, and the hind parts of the left ovaries are similar to those of the right ovary. However,

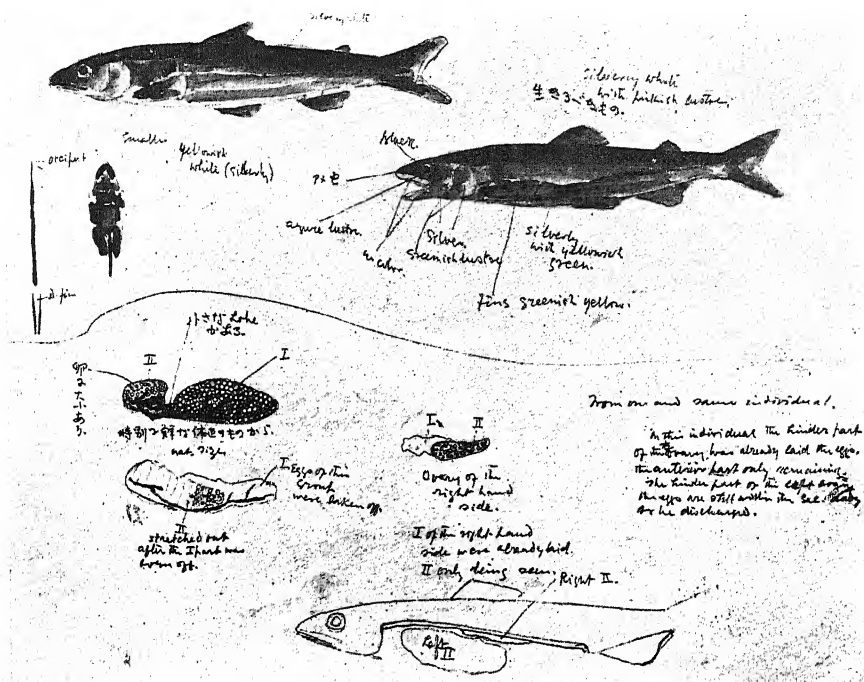
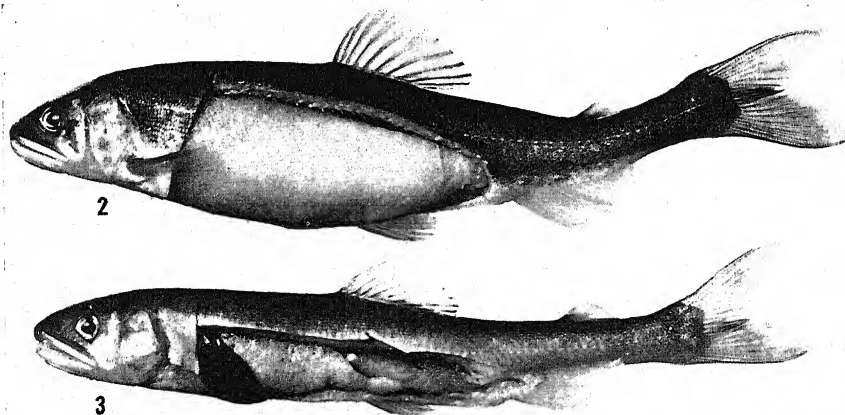


Fig. 1. The note of the observation of "Koayu", written by Dr. Ishikawa at Ungaiki Pond on 16th of November, 1928.

the anterior part of the left contains the immature eggs of white color. This fact corresponds well with the description of the note written by Ishikawa. In his description of November 16th, 1928, he says in Japanese that there are observed the eggs of larger and smaller size



Figs. 2-3. 2. Full matured ovary of the female at the beginning of the spawning season. 3. Immatured ovary of the female at the beginning of the spawning season.

in the fish which have pretty color with luster (fig. 1). It is very evident that different growth of the eggs is found between the anterior and the posterior parts of the ovary at the same time in the fish of Unagiike Pond, what so far was not observed in the fish of other places.

Looking over the results of collections in the river Tamagawa, regarding the growth of ovaries in specimens obtained at different stage of the breeding season, numerous fishes with immature ovaries were observed in the early stage, and they reduced to a very few at the final stage. At the middle stage, matured ovaries were observed in the majority of fish, and there was a decrease of such fish at the final stage. A few fish with detached ovaries were observed at the middle stage, and numerous of them were observed at the final stage.

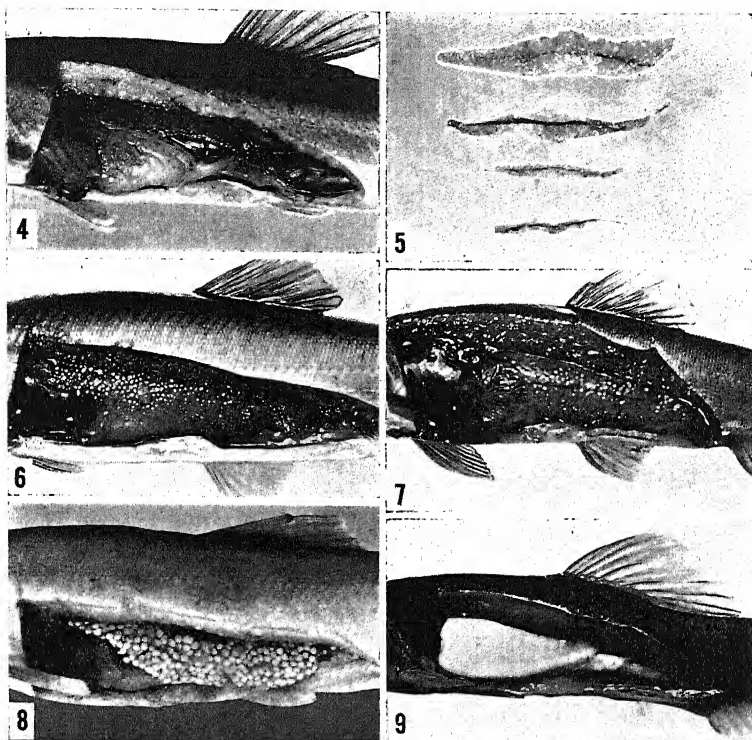
The gonads of "Otunen-Ayu" i.e. female fish which have passed over the breeding season, will be described in the following.

In the early stage of the B period, the majority of the fish which have already laid eggs are observed as shown in figs. 4 and 5. Owing to the detachment of the eggs, the whole ovary has decreased in size, resulting that the ovary-like cord are lying in the dorsal part of the body cavity. Some fish have not laid the eggs completely, and well riped eggs remain in the body cavity as shown in fig. 6. However there are observed some fish which have immature eggs in the ovaries as shown in fig. 7. The majority of the fish are found at the final stage of the period in which they have already detached the eggs, so that there remain a few eggs in the ovaries. In some fish, many eggs in the ovaries have undergone degeneration, as shown in fig. 8. All the specimens described above are found in the river Tamagawa. At the Unagiike Pond in the period B, the writer has obtained specimens with quite different appearance of the gonads compared with the specimens of other places.

In the specimen of the river Tamagawa there is found no nest of young oocytes such as are present in the ovary of the growing stage in the A period, and one might assume that the oocytes have changed to ova, which are not detached. The ova are filled with yolk, but the latter have various shades and are changed into vacuoles (fig. 10). Some of the ova have changed considerably, almost the whole yolk has degenerated, and a great part of the ova has become empty (fig. 11). The follicle cells of the ova have degenerated, and present many vacuoles (fig. 10). It is of remarkable evidence that the ova have produced the ring stained with dark shades on the inner part of the ova just beneath the follicle. The results of investigations of



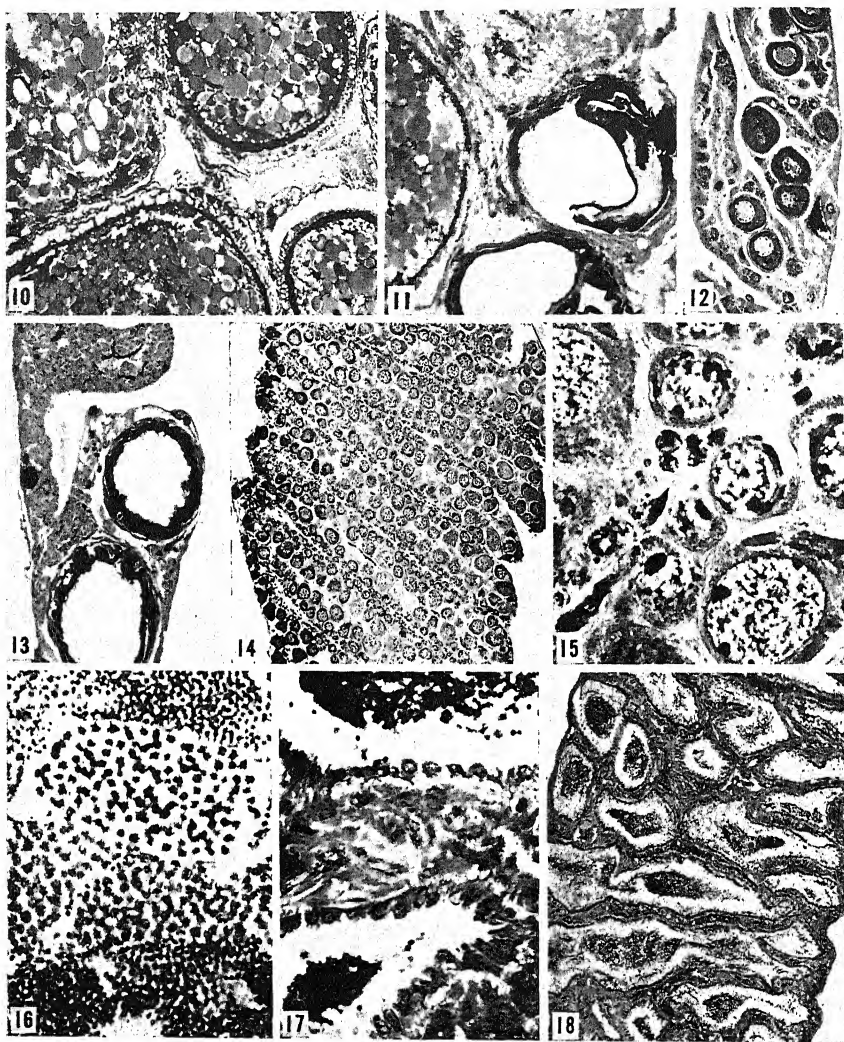
the ovary at this stage show well the degeneration of ova and no formation of a new nest of germ cells.



Figs. 4-9. 4. Atrophic ovary of the female after the spawning season, i.e. February 18th. 5. Ovaries of the specimens after the spawning season. 6. Ovary with well riped eggs in the female after the spawning season, i.e. March 19th. 7. Matured ovary of the female after the spawning season, i.e. February 18th. 8. Ovary with degenerated eggs in the female after the spawning season, i.e. March 19th. 9. Testis of the male in the middle stage of the spawning season.

Remarkable differences are observed in the specimens of the Unagiike Pond at this stage. The whole ovary has decreased markedly in size, so that the small and flattened ovary lies in the dorsal part of the body cavity. One or four oocytes that have grown considerably are found in the lobule, and oogonia and young oocytes in various stages of growth fill out its bulk (figs. 12 and 13). The most striking growth is observed in some oocytes in the lobule, which are very few number.

As mentioned in the specimen of the river Tamagawa, the degenerated ova can here also be seen in the lobule with nests of oogonia and oocytes (fig. 13). The nests of germ cells are found in peripheral region of the lobule, and the oogonial and oocyte cells formed the cysts in the lobules. Some of the oocytes in a cyst now



Figs. 10-18. 10. Section through ovary of fish, after the spawning season in the river Tamagawa. Ova fully formed and filled with degenerating yolk and vacuoles. 11. Section through ovary of fish of the same period as in fig. 10. Yolk of two ova are changed into vacuoles and yolk of another ovum are degenerating. 12. Section through ovary of fish after the spawning season at Unagiike Pond, showing one or two large oocytes in the lobule and the cytes of young germ cells in the peripheral region of one lobule. 13. Section through ovary of fish in the period as in fig. 12, showing degeneration of two ova and newly formed lobules. 14. Section through ovary of young fish at the end of April in the river Tamagawa, showing oocytes in the inner part of lobules, and oögonial cells in the peripheral region of one lobule. 15. Showing details of the germ cell in the ovary of fig. 14. 16. Section through anterior part of testis in the fish at the middle stage of the spawning season in the river Tamagawa. Outer cyst contains spermatogonia and inner cyst contains spermatozoa. 17. Section through posterior part of testis in the fish at middle stage of the spawning season. It is noted that there is observed only spermatozoa within lobules. 18. Section of the same testis, showing lobules with many spermatozoa, and Sertoli cells present in the inner border of the lobules.

enter a period of growth, so that we find cysts in which one or two cells are much greater than the rest.

In their size, form and staining reaction of their nuclei, the oogonia and oocytes in the nest resemble those of the peripheral regions of the lobules in the young fish in spring which is described as follows.

Figs. 14 and 15 show the sections through the ovary in young fish which ascended to the river from the sea in spring. Some of the oocytes have grown considerably, and have formed separated clusters in the center of the lobule in the ovary. The oogonia and young oocytes are found on the peripheral region of the lobule, which are very few in number, compared with oocytes in the inner part of the lobule.

As foregoing facts show the writer has observed, by means of examining gonads, two types in the specimen after their breeding season. One type produces a new crop of germinal cells in the ovary, and the other type yields no new crop of them having enlarged ova, which seem to have matured later in the B period.

### Male gonads

Now, in order to know the nature of the two types in the life history of the fish, the appearance and the fate of the germ cells in the gonads from the male fish obtained at different stages are examined by means of microscopical studies.

The appearance and relationship of the fish taken in the spawning season are shown in their aspect in fig. 9. Each testis is typically flat, somewhat concave and usually the left testis is very larger. The sperm duct connects with the inside of the testis, and it is filled with seminal fluid like milky, so that the seminal fluid came out on squeezing in the prime of the spawning season. The entire testis is covered with peritoneum. At the growth stage, the capsule consists of a very thin membrane. However, on the latter stage of the spawning season, the capsule gradually increases in thickness. The testis of the Ayu fish is composed of seminiferous lobules as usual in fish. The testes are divided by the trabeculae of the capsule into the lobules. The lobules are in turn subdivided into cysts by secondary septa.

The observation of the process of the germ cell formation in the testicular lobules during the spawning season are summarized in the following description and figures. In mature individuals it is variable in different regions of the testis.

As shown in fig. 16, the lobules consist of the cysts of the germ

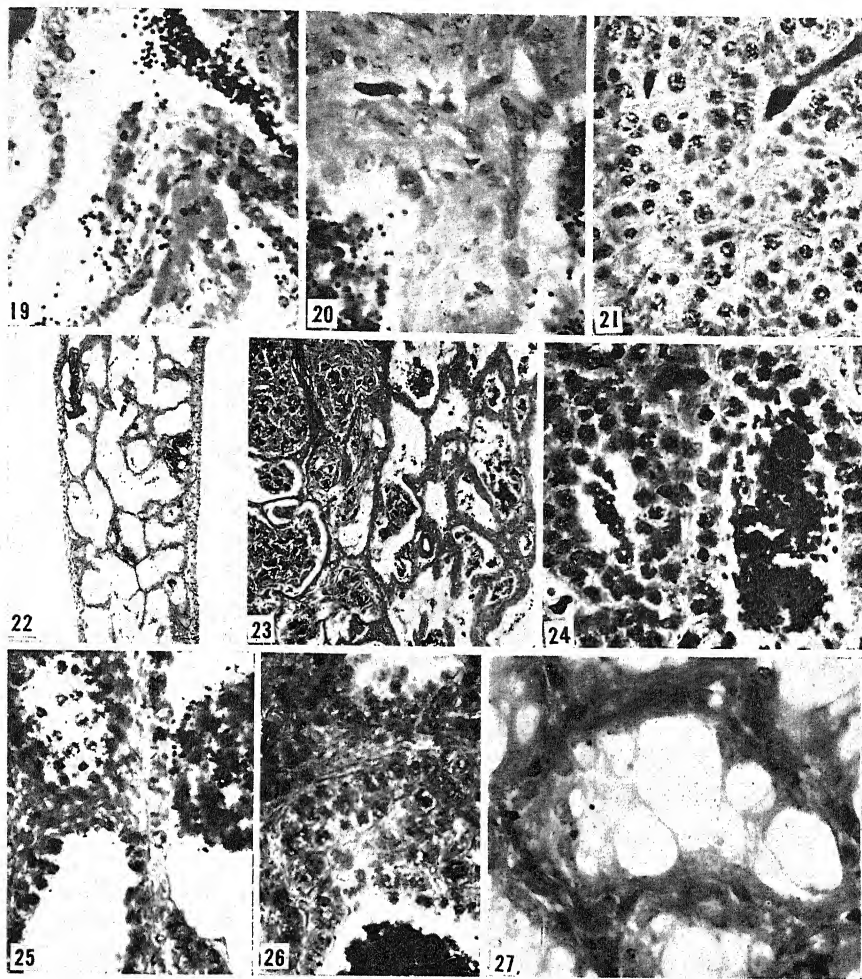
cells in the different stages at the anterior part of the testis. The mature spermatozoa being situated in the center part of the lobules, while the spermatogonial cells appear in the cysts at their periphery. In many individuals obtained during the spawning season the process of spermatogenesis in the lobules at the anterior part is similar to the above mentioned facts. The testicular lobules at the posterior of the testis represent the most striking evidence. The lobules are filled with mature spermatozoa ready to be expelled, and the Sertoli cells are arranged around the inside of the lobules, as seen in figs. 17 and 18. It will be seen that clumps of the spermatozoa are surrounded by a single layer of Sertoli cells. The Sertoli cells are separated from the inner border of the lobule, and are projecting toward the center of the lobules (fig. 19).

An interesting fact is observed in the specimens obtained in the middle of October. Fig. 20 shows a lobule just before the expulsion of the spermatozoa, containing a few large resting spermatogonial cells lining the inner border of the lobule. Such spermatogonial cells are similar in size, shape, and staining reaction to those in gonads of the young Ayu fish obtained in April (fig. 21). It might be conjectured that these spermatogonia found inside of the lobules are the product of the germ cells which has been referred to by other investigators as 'reserve germ cells' from an earlier generation. But, in the later examinations, it was shown that new germ cells may arise from the storoma cells lining the testicular lobule wall, and there is a possibility that the germ cells shown in figure may represent new germ cells and no residual cells have existed in the lobule from an early development. At the end of the spawning season, December 11th, the writer has obtained fish from the river Tamagawa just before they died. The testicular lobules show markedly changed features, and only a few spermatozoa are observed in the lobules (figs. 22 and 27). A few Sertoli cells lie scattered around the inside of the lobules and the separated Sertoli cells from the inside of the lobule seem to be degenerated in the interior of the lobule.

The male fish are not found among the collections from the river Tamagawa from December 11th, wherefore the 10 male fish are obtained at the Samezima fish firm on the 31st of January.

Shape and size of the testis of the male fish are similar to those of the fish during the spawning season. However, the results of microscopical examination of testis are of marked different. The testis is composed of the changed lobules and the empty ones, and the young germ cells in the cysts are degenerated (fig. 23).

At the end of March, after the spawning season, the marked features of the regeneration in the testis are observed in the fish



Figs. 19-27. 19. Section of the same testis as in fig. 18, showing lobules with many spermatozoa and Sertoli cells lining in the inner border of the lobules and projecting toward the center in one lobule. 20. Section through testis of fish at the middle stage of the spawning season in the river Tamagawa. It is observed that a few of spermatogonia in the inner border of the lobule are formed newly. 21. Section through testis of young fish at the end of April, showing cysts of the spermatogonia. 22. Section through testis of fish at the end of the spawning season, showing changed lobules. Some lobules contain only a few of the spermatozoa. 23. Section through testis of fish obtained at the fish firm, showing degenerating cysts in the lobules. 24. Section through testis of fish after the spawning season at Unagiike Pond, showing newly formed spermatogonial cells in the inner border of the lobules. The clumps of the spermatozoa are degenerated in the center of the lobule. 25, 26. Sections of the same testis, showing newly formed spermatogonial cells in lobules. The spermatozoa fill interior of the lobules. 27. Section of the same testis, showing the empty lobule. In one lobule the separated Sertoli cells formed inside of the lobule are projecting toward the center of the lobule.

obtained at Unagiike Pond. Fig. 25 shows that the spermatogonial cells are arranged around the inner border of the lobules before the discharge of the spermatozoa. Then the lobules of the testis are gradually filled with an accumulation of germ cells (figs. 24, 26).

Looking over the results of the present examination on the lobules in the testis during and after the spawning season, the changes of the lobules in the male can be divided into the following two types.

\* In the first type the new crop of germ cells is not found in the lobules after the expulsion of the spermatozoa, the lobules being composed of empty and degenerating cysts. In the second type, a new crop of germ cells is formed within the previously existing lobules, as it is the case of other organism in the seasonal cycle.

### Discussion

The studies regarding the origin of germ cells in the various adult organisms which well produce the annual crop of germ cells in the seasonal regeneration of the gonads, are reported by the several investigators.

Turner ('19), working on the adult perch, asserts that the annual crop of spermatogonia comes from cells, which enters the empty lobules. In his studies on the germ cell history of the adult *Diemyctylus*, Hagitt ('24) reported that germ cells in the seasonal regeneration of the gonad arise from outside the cyst. Foley ('27) claims that in *Umbra limi* the spermatogonia arise mainly from transformation of stroma cells of the testis which, in the process, may migrate into previously existing lobules. In addition, some transform themselves in the fibrous tissue of the testis.

The present work has been made as an effort to ascertain whether the phenomenon of new crop formation of germ cells is also found in the gonad of *P. altivelis* in the period after the spawning season.

As stated in the previous pages, a careful examination of the germ cells in the gonads of the female and male fish and an appearance of the gonads was made from the end of September to the middle of June. In the female Ayu fish, the specimens with matured ovary are observed in the majority of the collections in the middle stage of the breeding season in the river Tamagawa, and they are gradually reduced in number till the end of the season.

It is seen that in specimens found in the earlier period after the breeding season, some showed matured ova as seen in the breeding season. But, in three months after the breeding

season, specimens with fully matured are entirely absent and almost all specimens had the cord-like ovary, or showed evidence of degenerated ova in the shrunken ovary. The results of the microscopical examination of these specimens show that nests of young germ cells are not produced in the ovary but degeneration of the ova is seen. In the specimens of Unagiike Pond, however, some fish were found to have newly formed nests of germ cells, while the other showed only degenerated large ova in their ovary.

The specimens after their breeding season, therefore, have two types in the appearance of the gonads:

One type has the production of new germ cells in the ovary and in the other type, there is no new crop of germ cells formed, and there remain the well grown ova in the early stage of the period and the ova accompanying degeneration of the yolk are observed in the late stages.

In the male Ayu fish, the specimens which have passed over the spawning season are not obtained in the river Tamagawa. Therefore, the appearance of the germ cell in the gonads can not be observed. But, at the end of the spawning season, the specimens were obtained which show the testis composed of the changed lobules and empty ones, and young germ cells are not found in the cysts of the lobules.

Almost all the specimens obtained after the spawning season at the fish farm have evidence of degenerated germ cells in the lobules of the gonads. At the Unagiike Pond, the marked features of the regeneration in the testis are observed in the specimens after the spawning season. It can be seen that the lobules of the testis are gradually filled with an accumulation of the young germ cells.

The appearance of the gonads in the specimens after the spawning season is not the same in the female and the male Ayu fish. It was not observed the survival male fish after the spawning season in the river Tamagawa, which the writer might assume that all the fish died soon after they have finished the discharge of spermatozoa while in the specimens at the Unagiike Pond regeneration in the testis was occurred as in the case of *Umbra* reported by Foley ('27).

### Summary

1. The appearance and fate of the germ cells in the gonads of the Ayu fish obtained during and after their spawning season in the rivers and the pond were studied.

2. In the river Tamagawa, the female fish after the spawning season could be obtained until in the middle of June, while the male

fish could not be obtained after the last October, except only two which were got until December (cf. table 1).

3. In the female fish of the river Tamagawa, there was no formation of a new crop of germ cells in the gonads, while it was observed in the male.

4. In the fish obtained at Unagiike pond, the new crop of germ cells was found to form in the gonads of both sexes after the spawning season.

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**Cyto-genetical Studies on *Tricyrtis*, II. Karyotype analysis in  
*Tricyrtis* and *Brachycyrtis* with special reference to SAT-  
and nucleolar chromosomes<sup>1)</sup>**

By

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*Received April 20, 1939*

**Introduction**

Cytological studies on *Tricyrtis* have been reported by many investigators (cf. Guignard 1884; Ikeda 1901, 1902; Ishikawa 1916; Nawa 1928, Miller 1930; Sinotô and Kikkawa 1932; Matsuura 1935; Matsuura and Sutô 1935; Satô 1937) and some earlier workers inaccurately stated the haploid number of this genus to be 14–16 (cf. Guignard 1884) or 12–13 (cf. Ishikawa 1916), but the other later workers are in agreement with the present observation concerning the chromosome number i.e.,  $n = 13$ ,  $2n = 26$ .

The reports on the karyotypes of this genus were also not sufficiently adequate, with the exception of that made in the previous paper of the present writer (Satô 1937); that is, the SAT-chromosomes have been overlooked by all previous investigators except Miller (1930) who found only one pair of short chromosomes with a satellite in *Tricyrtis macropoda*, *T. pilosa* and *T. stolonifera*. Matsuura and Sutô (1935) described the idiogram in *T. latifolia* overlooking the SAT-chromosomes, but they found the secondary constriction in two pairs of long chromosomes. The present work was partly undertaken to clarify the cause of such confusion as regards the absence or presence of SAT-chromosomes. Consequently, the karyotypes of *Tricyrtis* were analysed from the view point of the variability of the SAT-chromosomes, such variation of the SAT-chromosomes having been reported on rare occasions in *Crepis* (cf. Swezy 1935), *Narcissus* (cf. Fernandes 1935) and *Galanthus* (cf. Satô 1937).

With respect to the formation of nucleoli, Heitz (1931a, b) has advanced the SAT-chromosome theory which implies that the nucleolus originates in the telophase in a certain region of the SAT-chromosomes, i.e., in the satellite stalk or secondary constriction, and that the number, position and size of the nucleoli correspond to those

1) Contributions from the Divisions of Genetics and of Plant-Morphology, Botanical Institute, Faculty of Science, Tôkyô Imperial University, No. 227.

of these regions of SAT-chromosomes present in the nuclei. Later this theory of Heitz was modified to reconcile it with the conception of the nucleolar chromosome without satellite or secondary constriction present in *Zea mays* (cf. McClintock 1934), *Trillium kamtchaticum* (cf. Matsuura 1935, 1938) and *Narcissus bulbocodium* var. *genuinus* (Fernandes 1936) etc. With the modified hypothesis as a basis "differential amphiplasty" in species hybrids of *Crepis*, viz. the disappearance of the satellite of one parental chromosomal complement in  $F_1$  hybrids (cf. Navashin 1934) is intelligibly interpreted by McClintock (1934). This work was undertaken with the object of investigating the relation between the nucleoli and SAT-chromosomes in many species and hybrids of *Tricyrtis* and *Brachycyrtis*. The present paper deals with the observation that the nucleolar chromosomes are mobilized as a substitute for SAT-chromosomes when some satellites have disappeared, and that disappearance of satellites occurs only in hybrids. These phenomena can be clearly explained on the basis of their being a differential rate in the capacity for nucleolus-forming activity of chromosomes (including SAT-chromosome, nucleolar chromosome and usual chromosome) within a complement as in *Zea* (cf. McClintock 1934) and *Trillium* (cf. Matsuura 1938).

### Materials and Methods

Most of the materials used were taken from plants collected and being kept by Prof. Sinotô in the Genetical Laboratory of the Botanical Institute, and the rest of them were obtained from plants cultivated in the Koisikawa Botanic Garden of the Tôkyô Imperial University. Many of the hybrids used in this study had been raised previously by Prof. Sinotô.

The root-tips were fixed with Navashin's fluid and occasionally with the Bonn modification of Flemming's solution. The paraffin sections were cut 15–20 micra in thickness and Newton's gentian violet method was used for staining. Sometimes Kaiser's fixative was also employed and Feulgen's nucleal reaction was tried for the purpose of identification of the SAT-chromosomes.

The pollen mother cells were fixed with Bouin's fluid by the ordinary paraffin method and the later procedures were the same as those followed in the case of the root-tips. The aceto-carmin smear method and the permanent smear method were also used, the fixative being either the Bonn modification of Flemming's solution or Navashin's fluid while the gentian violet method was used for staining in the latter case.

The pollen grains were observed principally by employing the aceto-carmin smear method. Sometimes the permanent smear method was used, the fixative being the Bonn modification of Flemming's solution or Kaiser's fluid and the staining being carried out according to Feulgen's nuclear reaction method.

### Observation

SAT-chromosomes in *Tricyrtis* were neglected and overlooked by the earlier investigators, for the satellites are very small and some satellite stalks are extremely short. For the reasons mentioned above some satellites can be found in favourable cases of observation, the term favourable here implying not only good fixation and staining but also favourable material or more strictly speaking favourable nuclei from the same or different root-tips of the same individual. According to the Heitz theory, the nucleoli in the telophase should first be counted carefully and then the corresponding SAT-chromosomes traced and when the correspondence between them is contrary to expectation, a more critical survey must be made of the surface of the nucleoli in the prophase. In such cases the mobilisation of the nucleolar chromosomes can be observed in addition to the SAT-chromosomes.

To analyse the karyotypes in *Tricyrtis*, the following abbreviations were adopted for the sake of convenience (cf. figs. 34, 35).

- L<sub>1</sub> One pair of long chromosomes with a subterminal constriction and also a subterminal secondary constriction at the long arm. Some species have satellites at the distal or proximal ends.
- L<sub>2</sub> One pair of long chromosomes with subterminal constriction and submedian secondary constriction at the long arm. Some species have satellites at the distal or proximal ends.
- S Eleven pairs of short chromosomes of which two pairs with subterminal constrictions sometimes have satellites at their proximal ends.

In the description of karyotypes the suffix 't' or 'n' is added on the shoulder of the above-mentioned symbols in order to distinguish the nucleolar chromosomes. The former designates a SAT-chromosome with a trabant and the latter a nucleolar chromosome without a trabant or a secondary constriction.

#### (I) Homozygous types of SAT-chromosomes in *Tricyrtis*

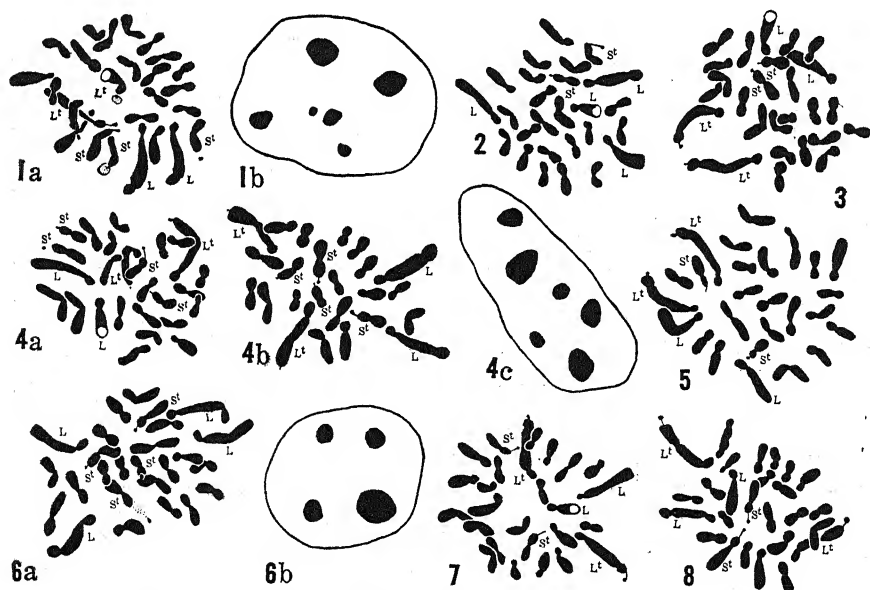
- 1) *T. formosana* var. *ramosa*  $2n = 26 = 2L_1 + 2L_2^t + 4S^t + 18S$   
(fig. 3)

This species has a typical chromosome complement i.e., two pairs of long chromosomes and eleven pairs of short ones ( $2n = 26 = 4L + 22S$ ). One pair of long chromosomes with subterminal secondary constriction ( $L_1$ ) is distinguished clearly from another pair of long chromosomes with submedian secondary constriction ( $L_2$ ) by the presence of a satellite at the distal end of the latter type. Two pairs of short chromosomes with subterminal constrictions also have satellites at their proximal ends. One of these pairs of short chromosomes has a large satellite and the other has a small one. The presence of six SAT-chromosomes was established, this number being in accordance with the six corresponding nucleoli in the telophase nucleus.

2) *T. affinis*  $2n = 26 = 2L_1 + 2L_2^s + 4S^t + 18S$  (fig. 1)

This species has also a typical chromosome complement and one pair of long chromosomes ( $L_2$ ) has a satellite at its proximal end while two pairs of short chromosomes also have satellites at their proximal ends.

3) *T. hirta* f. *variegata*  $2n = 26 = 2L_1 + 2L_2^s + 2S^t + 20S$  (fig. 8)



Figs. 1-8. Homozygous types of SAT-chromosomes in *Tricyrtis*. 1, *T. affinis*  $2n = 26 = 2L_1 + 2L_2^s + 4S^t + 18S$ . 2, *T. Yatabeana*  $2n = 26 = 2L_1 + 2L_2 + 2S^t + 20S$ . 3, *T. formosana* var. *amethystina*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$ . 4, *T. formosana* var. *ramosa*  $2n = 26 = 2L_1 + 2L_2^s + 4S^t + 18S$ . 5, *T. formosana* var. *kotoensis*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$ . 6, *T. dilatata*  $2n = 26 = 2L_1 + 2L_2 + 4S^t + 18S$ . 7, *T. "Taiwan 1"*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$ . 8, *T. hirta* f. *variegata*  $2n = 26 = 2L_1 + 2L_2^s + 2S^t + 20S$ .  $\times 1750$ .

This species has four SAT-chromosomes, that is, one pair of long chromosomes ( $L_2$ ) has a satellite at its distal end and one pair of short chromosomes has a satellite at its proximal end.

4) *T. formosana* var. *amethystina*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$  (fig. 3)

5) *T. formosana* var. *kotoensis*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$  (fig. 5)

6) *T. "Taiwan 1"*  $2n = 26 = 2L_1^t + 2L_2 + 2S^t + 20S$  (fig. 7)

These species have four SAT-chromosomes, that is, one pair of long chromosomes ( $L_1$ ) has a satellite at the distal end and one pair of short chromosomes also has a satellite at its proximal end.

7) *T. dilatata*  $2n = 26 = 2L_1 + 2L_2 + 4S^t + 18S$  (fig. 6)

This species has 26 chromosomes of which two pairs of short chromosomes have satellites at their proximal ends. This species differs from the other species, with the exception of *T. Yatabeana*, *T. formosana* and *T. perfoliata*, in having no long SAT-chromosome.

8) *T. Yatabeana*  $2n = 26 = 2L_1 + 2L_2 + 2S^t + 20S$  (fig. 2)

This species has 26 chromosomes of which one pair of short chromosomes has a satellite at the proximal end. This species also has no long SAT-chromosome. It is interesting to note that the chromosome complement of this species is shorter than those of the other species with many satellites, but longer than that of *T. perfoliata* with no satellite (cf. figs. 19, 34, 35).

## (II) Heterozygous types of SAT-chromosomes in *Tricyrtis* and *Brachycytis*

9) *T. hirta*  $2n = 26 = 2L_1^t + 1L_2^t + 1L_2 + 3S^t + 19S$  (fig. 9)

This species has 26 chromosomes of which one pair of long chromosomes with subterminal secondary constriction ( $L_1$ ) has a satellite at the proximal end, one long chromosome ( $L_2$ ) has a satellite at its distal end, one short chromosome has a small satellite at its proximal end and two short chromosomes have large satellites at their proximal ends. Six SAT-chromosomes have been detected in the prophase and metaphase and correspond to the six nucleoli in the telophase. One long chromosome ( $L_2$ ) and one short chromosome have no homologous chromosomes with satellite and this suggests the translocation of the satellite.

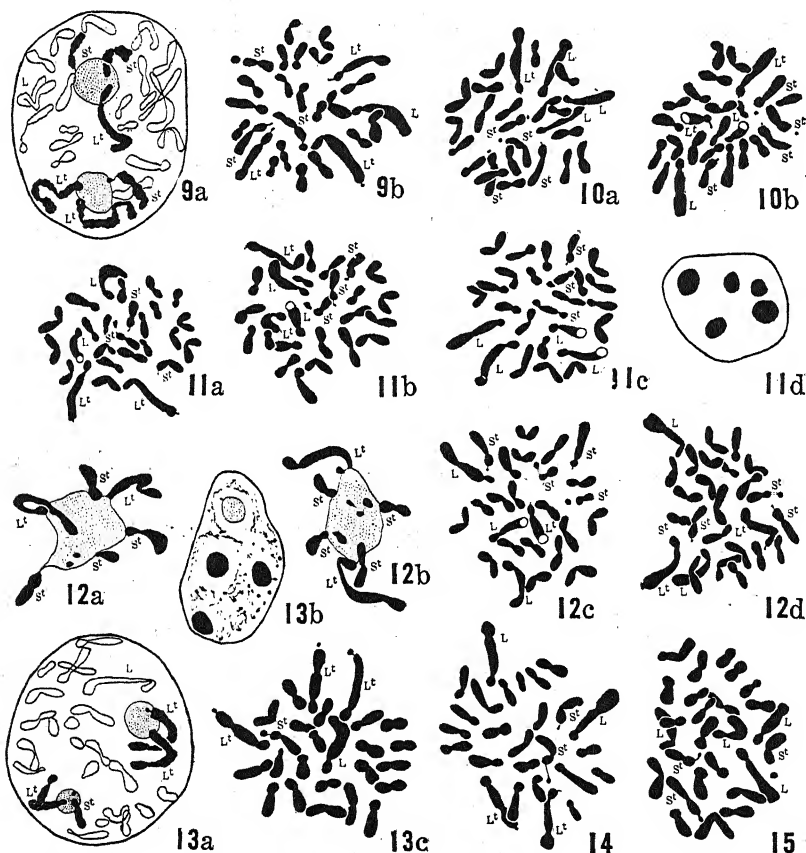
10) *T. hirta* from the Koisikawa Botanic Garden  $2n = 26 = 2L_1^t + 2L_2 + 4S^t + 18S^t$  (fig. 10).

This species has 26 somatic chromosomes of which one pair of long chromosomes ( $L_1$ ) and two pairs of short chromosomes have satellites at their proximal ends. This plant has homozygous SAT-

chromosomes and, when compared with the plant in the Genetical Laboratory mentioned above, appears to be an original type.

- 11) *Brachycyrtis macrantha*  $2n = 26 = 2L_1^t + 2L_2 + 3S^t + 19S$  (fig. 11)

This species has five SAT-chromosomes, namely one pair of long chromosomes ( $L_1$ ) has an extremely small satellite at the distal end and three short chromosomes have satellites at their proximal ends. In other individual there were only three SAT-chromosomes of which one pair of short chromosomes has a large satellite at its proximal end and one short chromosome has a small satellite at its proximal end. This picture also suggests the structural



Figs. 9-16. Heterozygous types of SAT-chromosomes in *Tricyrtis* and *Brachycyrtis*. 9, *T. hirta* from Genetical Laboratory  $2n = 26 = 2L_1^t + 1L_2^t + 1L_2 + 3S^t + 19S$ . 10, *T. hirta* from Koisikawa Botanic Garden  $2n = 26 = 2L_1^t + 2L_2 + 4S^t + 18S$ . 11, *Brachycyrtis macrantha*  $2n = 26 = 2L_1^t + 2L_2 + 3S^t + 19S$ . 12, *B. macranthiopsis*  $2n = 26 = 2L_1^t + 2L_2 + 4S^t + 18S$ . 13, *T. formosana* var. *lasiocarpa*  $2n = 26 = L_1^t + L_1 + 2L_2^t + S^t + 20S$ . 14, *T. hirta* f. *albo-marginata*  $2n = 26 = 1L_1^t + 1L_1 + 1L_2^t + 1L_2 + 2S^t + 20S$ . 15, *T. hirta* f. *albida*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ .  $\times 1750$ .

hybridity of the SAT-chromosomes and a satellite deficiency in the long chromosomes.

- 12) *Brachycyrtis macranthiopsis*  $2n = 26 = 2L_1^t + 2L_2 + 4S^t + 18S$  (fig. 12)

This species has 26 somatic chromosomes of which one pair of long chromosomes ( $L_1$ ) and two pairs of short chromosomes have clearly defined satellites at the metaphase (fig. 12 c, d) and become attached to the nucleolus at the prophase (fig. 12 a, b). One pair of short SAT-chromosomes has large satellites and another pair has comparatively large satellites, both at the proximal ends. One long SAT-chromosome has a small satellite at its proximal end and another one has a comparatively large satellite at its distal end. This suggests a translocation of the satellite.

- 13) *T. formosana* var. *lasiocarpa*  $2n = 26 = L_1^t + L_1 + 2L_2^t + S^t + 21S$  (fig. 13)

This species has four SAT-chromosomes and the same number of nucleoli of which three are in one plane while the other is in a different plane. One pair of long chromosomes with submedian secondary constriction ( $L_2$ ) has small satellites at its distal end, one short chromosome has a large satellite at its proximal end and one long chromosome ( $L_1$ ) has a large satellite at its distal end. These heterozygous SAT-chromosomes suggest the translocation of the satellite between the long ( $L_1$ ) and the short chromosomes.

- 14) *T. hirta* f. *albida*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$  (fig. 15)

This species has three short chromosomes with satellites which resemble those of *T. hirta*.

- 15) *T. hirta* var. *albo-marginata*  $2n = 26 = 1L_1^t + 1L_1 + 1L_2^t + 1L_2 + 2S^t + 20S$  (fig. 14)

This species has four SAT-chromosomes, namely one long chromosome ( $L_1$ ) has a satellite at its proximal end, one long chromosome ( $L_2$ ) has a satellite at its distal end and two short chromosomes have satellites at their proximal ends. This karyotype is interesting because of the contrast it presents with that of *T. hirta* f. *variegata*, which has two long chromosomes ( $L_2$ ) with satellite at their distal ends and two short chromosomes with satellite at their proximal ends. Consequently we may conclude that the long chromosome with a satellite at its proximal end ( $L_1$ ) is a result of translocation of the satellite from the distal end of the long chromosome ( $L_2$ ).

### (III) Variability of SAT-chromosomes in *Tricyrtis*

- 16) *T. latifolia*  $2n = 26 = 2L_1 + 2L_2^t + 2S^t + 20S$  (fig. 16 d-m)  
 $2n = 26 = 2L_1 + 1L_2^t + 1L_2 + 2S^t + 20S$  (fig. 16 a-c)

This species was reported, in my previous paper, (cf. Satô 1937), to have three SAT-chromosomes but careful observation has shown that most metaphase plates have three SAT-chromosomes, while in a few cases there are four SAT-chromosomes. These four SAT-chromosomes consist of one pair of long chromosomes with submedian secondary constriction ( $L_2$ ) having satellites at the proximal end and one pair of short chromosomes having satellites at the proximal end. One long SAT-chromosome is found commonly

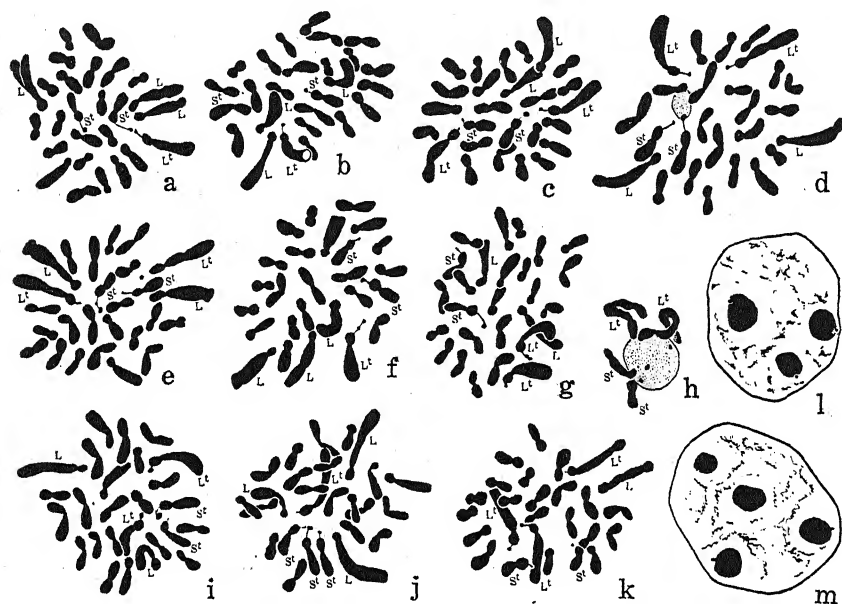


Fig. 16. Variation of SAT-chromosomes in *Tricyrtis latifolia*. a-c, Individual with three SAT-chromosomes ( $1L_2^t + 2S^t$ ). c, Four satellites are seen in the same root-tip cells. Notice an extremely small satellite of one long chromosomes. d-m, Individual with four SAT-chromosome ( $2L_2^t + 2S^t$ ). One short chromosome has a small satellite and other SAT-chromosomes have large satellites. e-g, i, Translocation of satellites between these SAT-chromosomes and tandem satellites are made at the expense of other satellites. j, h, Translocation of satellites between SAT-chromosomes and non-homologous chromosomes. l, m, nucleoli at the resting stage. Notice the small deeply stained bodies or satellites on the surface of nucleoli. l, Three nucleoli, one of them is large and may be derived from the fusion of two. m, Four nucleoli.  $\times 1750$ .

in all metaphase plates, but its homologous chromosome has no satellite in most cases and three nucleoli are counted in the telophase nucleus. Another individual belonging to this species has four satellited chromosomes, namely one pair of long chromosomes ( $L_2$ ) with a satellite at the proximal end and one pair of short chromosomes with satellites at the proximal end of which one chromosome has a large satellite and the remaining one a small satellite.



The satellite sizes of these four SAT-chromosomes change and sometimes tandem satellites are formed at the expense of the other satellite. For instance short chromosomes with a tandem satellite are derived from translocation of the satellite belonging to the long chromosome (fig. 16 e) or the homologous short chromosome (fig. 16 i) and long chromosomes with tandem satellites are also formed by the translocation of the satellite belonging to the homologous long chromosome (fig. 16 f and g). Such a translocation seems to occur in the prophase, when these SAT-chromosomes become attached to one fused nucleolus (fig. 16 h) and then separate from one another as a result of the dissolution or disappearance of the nucleolus. Besides these cases of translocation of the satellite between the SAT-chromosomes the satellite is translocated to other non-homologous chromosomes in rare cases (fig. 16 j, k).

With regard to the nucleoli the present observation clearly establishes the existence of the corresponding number of four (fig. 16 l) and rarely three in the telophase. In the latter case one nucleolus is large and may be derived from the fusion of two nucleoli (fig. 16 l). A deeply stained body or satellite is expected, in the light of the hypothesis of SAT-chromosomes, to exist on the surface of each nucleolus, but only three instead of four were observed in the present case and these three heterochromatic bodies perhaps correspond to three large satellites in this species (fig. 16 m).

Such variation of the SAT-chromosomes is observed in many other species of *Tricyrtis* and *Brachycyrtis*, such as *T. hirta*, *T. formosana* var. *stolonifera* and *T. formosana* var. *amethystina* and *B. macrantha*, and various individuals with different combinations of SAT-chromosomes are found in the same species (cf. *T. hirta* and *T. formosana*) as in *Narcissus reflexus*, *N. triandrus* (cf. Fernandes 1935), *Scilla permixta* and *S. ughii* (cf. Satô 1936).

#### (IV) SAT-chromosomes and nucleolar chromosomes in *Tricyrtis*

The foregoing paragraph contains convincing evidence that the number, position and size of the nucleoli in the telophase correspond to those of the SAT-chromosomes in various species of *Tricyrtis*. Heterozygous types of SAT-chromosomes and also the variation of SAT-chromosomes suggest translocation and elimination of the satellites, and hence the presence of nucleolar chromosomes. Consequently the following three combinations may be classified, 1) SAT-chromosomes alone, 2) SAT-chromosomes and nucleolar chromosomes and 3) nucleolar chromosomes alone.

- 17) *T. formosana* var. *stolonifera*  $2n = 26 = 2L_1^t + 2L_2^t + 2S^t + 20S$  (fig. 17 c-h)

$$2n = 25 = 2L_1^t + 2L_2^t + 1M + 2S^t + 18S \text{ (fig. 17 a, b)}$$

Two different individuals are distinguished in this species, one having 25 somatic chromosomes and the other 26 somatic chromosomes. The 25-chromosomic individual has five long chromosomes and twenty short chromosomes. Of the five long chromosomes one pair of chromosomes has a subterminal secondary constriction ( $L_1$ ) with a small satellite at the distal end, another pair has a submedian secondary constriction ( $L_2$ ) and remaining one (M) has three constrictions and may be derived from the fusion of two short chromosomes (fig. 17 a). One pair of short chromosomes has large satellites at the proximal end. In the prophase two short chromosomes with large satellites and two long chromosomes become attached to the nucleoli (fig. 17 b). Four SAT-chromosomes and nucleoli are easily detected in this individual.

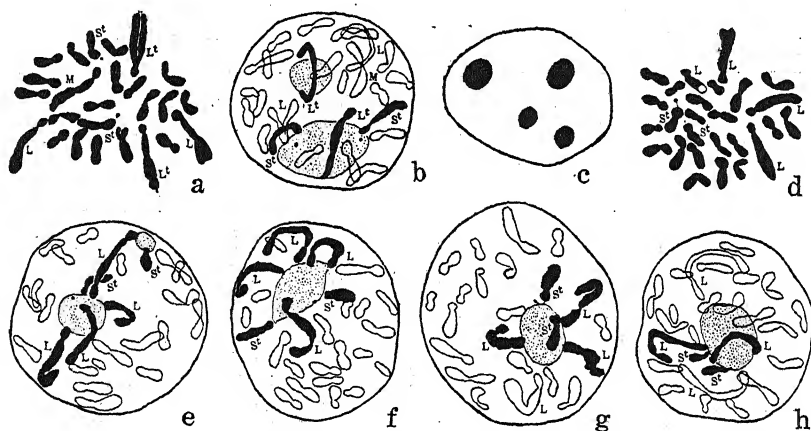


Fig. 17. SAT-chromosomes and nucleolar chromosomes in *Tricyrtis formosana* var. *stolonifera*. a, b, *T. formosana* var. *stolonifera* no. 2.  $2n = 25 = 2L_1^t + 2L_2^t + 1M + 2S^t + 18S$ . c-h, *T. formosana* var. *stolonifera* no. 1.  $2n = 26 = 2L_1^t + 2L_2^t + 2S^t + 20S$ .  $\times 1750$ .

Another individual having 26 somatic chromosomes was previously reported to have only two short chromosomes with satellites in spite of the presence of four nucleoli in the telophase (fig. 17 c) (cf. Satô 1937c). One short chromosome has a large satellite and another short chromosome has a small satellite and these two chromosomes become attached to the nucleoli in the prophase. Besides the two SAT-chromosomes two long chromosomes ( $L_1$ ) become attached to a nucleolus (fig. 17 h) and sometimes four long chromosomes ( $L_1$  and  $L_2$ ) become attached to the nucleoli (fig. 17 e, f).

In most cases four chromosomes, namely two SAT-chromosomes and two long chromosomes ( $L_1$ ) become attached to nucleoli as in the other individual, but it is interesting to note that another long pair ( $L_2$ ) also become attached to the nucleolus at its proximal end.

18) *T. formosana*  $2n = 26 = 2L_1^+ + 2L_2^+ + 1S^+ + 21S$  (fig. 18)

This species has 26 somatic chromosomes in which one short chromosome has a satellite at its proximal end, while one large and three small nucleoli are found in the telophase (cf. Satô 1937c).



Fig. 18. SAT-chromosome and nucleolar chromosomes in *Tricyrtis formosana*  $2n = 26 = 2L_1^+ + 2L_2^+ + 1S^+ + 3S^n + 18S$ .  $\times 1750$ .

To clarify this contradiction of the relation between SAT-chromosomes and nucleoli, attention was concentrated on the surface of the nucleoli in the prophase. One short SAT-chromosome becomes attached to the nucleolus in all observed cases and three long chromosomes ( $2L_1 + L_2$ ) also become attached to the nucleolus (fig. 18 g) in many cases. More detailed observation showed that eight chromosomes become attached to nucleoli in the prophase, namely one pair of long chromosomes ( $L_1$ ) becomes attached to nucleoli at the distal ends, another pair of long chromosomes ( $L_2$ ) at the proximal ends and two pairs of short chromosomes at the proximal ends (fig. 18 a-c). It is interesting to note that particular regions of particular chromosomes become attached to the nucleoli in the prophase, even though only one SAT-chromosome and four nucleoli are found.

19) *T. perfoliata*  $2n = 26 = 4L + 4S^n + 18S$  (fig. 19)

This species has the smaller chromosomes in this genus. No SAT-chromosome is found in all 26 somatic chromosomes (fig. 19 i), while four nucleoli are clearly observed in the telophase (fig. 19 h).

Four short chromosomes become attached to the nucleoli (fig. 19 f, g) in many prophase nuclei and sometimes other chromosomes including long ones also become attached to the nucleoli, in addition to these four short chromosomes (fig. 19 a-e). These chromosomes attaching to the nucleoli vary in number from four to ten and the long chromosomes seem, as far as the present observation is concerned, to become attached to the nucleoli at their proximal ends.

Table 1. Comparison of karyotypes in *Brachycyrtis* and *Tricyrtis*

Species name (Plant no.)	Karyotypes (2n)	Nucleoli
1. <i>Brachycyrtis</i>		
<i>B. macrantha</i> (7) <sup>1)</sup>	$26 = 2L_1^t + 2L_2 + 3S^t + 19S$	5
<i>B. macranthiopsis</i> (45)	$26 = 2L_1^t + 2L_2 + 4S^t + 18S$	6
2. <i>Tricyrtis</i>		
Sect. Tamagawa		
<i>T. latifolia</i> no. 1 (6)	$26 = 2L_1 + 2L_2^t + 2S^t + 20S$	4
<i>T. latifolia</i> no. 2 (6)	$26 = 2L_1 + 1L_2^t + 1L_2 + 2S^t + 20S$	3
Sect. Flavae		
<i>T. Yatabeana</i> (2)	$26 = 2L_1 + 2L_2 + 2S^t + 20S$	2
<i>T. perfoliata</i> (46)	$26 = 4L + 4S^t + 19S$	4
Sect. Hirtae		
<i>T. hirta</i> no. 1 (4)	$26 = 2L_1^t + 1L_2^t + 1L_2 + 3S^t + 19S$	6
<i>T. hirta</i> no. 2 (4)	$26 = 2L_1^t + 2L_2 + 4S^t + 18S$	6
<i>T. hirta</i> f. <i>albida</i> (12)	$26 = 2L_1 + 2L_2 + 3S^t + 19S$	3
<i>T. hirta</i> f. <i>variegata</i> (16)	$26 = 2L_1 + 2L_2^t + 2S^t + 20S$	4
<i>T. hirta</i> f. <i>albo-marginata</i> (41)	$26 = 1L_1^t + 1L_1 + 1L_2^t + 1L_2 + 2S^t + 20S$	4
Sect. Maculata		
<i>T. formosana</i> (13)	$26 = 2L_1^n + 2L_2^n + S^t + 3S^n + 18S$	4
<i>T. formosana</i> var. <i>stolonifera</i> No. 1 (9)	$26 = 2L_1^n + 2L_2^n + 2S^t + 20S$	4
<i>T. formosana</i> var. <i>stolonifera</i> No. 2 (9)	$25 = 2L_1^t + 2L_2 + 1M + 2S^t + 18S$	4
<i>T. formosana</i> var. <i>lasiocarpa</i> (5)	$26 = 1L_1^t + 1L_1 + 2L_2^t + S^t + 21S$	4
<i>T. formosana</i> var. <i>amethystina</i> (42)	$26 = 2L_1^t + 2L_2 + 2S^t + 20S$	4
<i>T. formosana</i> var. <i>kotoensis</i> (43)	$26 = 2L_1^t + 2L_2 + 2S^t + 20S$	4
<i>T. formosana</i> var. <i>ramosa</i> (44)	$26 = 2L_1 + 2L_2^t + 4S^t + 18S$	6
<i>T. "Taiwan 1"</i> (37)	$26 = 2L_1^t + 2L_2 + 2S^t + 20S$	4
Sect. Macropoda		
<i>T. affinis</i> (1)	$26 = 2L_1 + 2L_2^t + 4S^t + 18S$	6
<i>T. dilatata</i> (10)	$26 = 2L_1 + 2L_2 + 4S^t + 18S$	4

1) 7,45, etc. = S32.7, S32.45, etc.

Even when the SAT-chromosome is completely absent, the nucleoli may be formed regularly by the mobilisation of nucleolar chromosomes as a substitute for SAT-chromosomes. The present observation also throws some light on the nature of nucleolar chromosomes in comparison with SAT-chromosomes, for these nucleolar chromo-

somes have no satellites, but can fulfil the same function in the formation of the nucleolus as the SAT-chromosomes in other species of *Tricyrtis* (cf. McClintock 1934 in *Zea mays*, Matsuura 1935, 1938 in *Trillium kamtschaticum*, Fernandes 1936 in *Narcissus bulbocodium* var. *genuinus*, etc.).

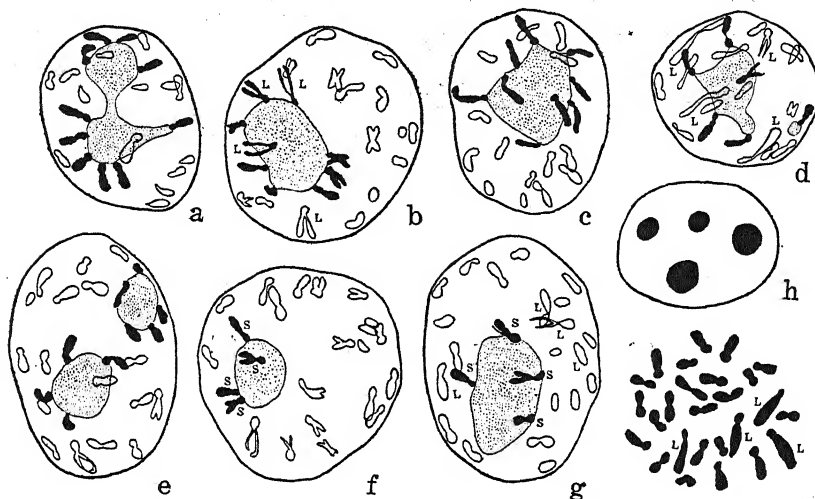


Fig. 19. Nucleolar chromosomes in *Tricyrtis perfoliata*  $2n = 26 = 4L + 4S^n + 18S$ .  $\times 1750$ .

#### (V) SAT-chromosomes of hybrids in *Tricyrtis* and *Brachycyrtis*

Heterozygous SAT-chromosomes were found in many species, so the hybrids of these plants have also heterozygous SAT-chromosomes.

##### 1) *T. hirta* $\times$ *T. formosana* var. *stolonifera*

$$2n = 26 = 1L_1^+ + 1L_1 + 2L_2 + 2S^t + 20S \quad (\text{fig. 20})$$

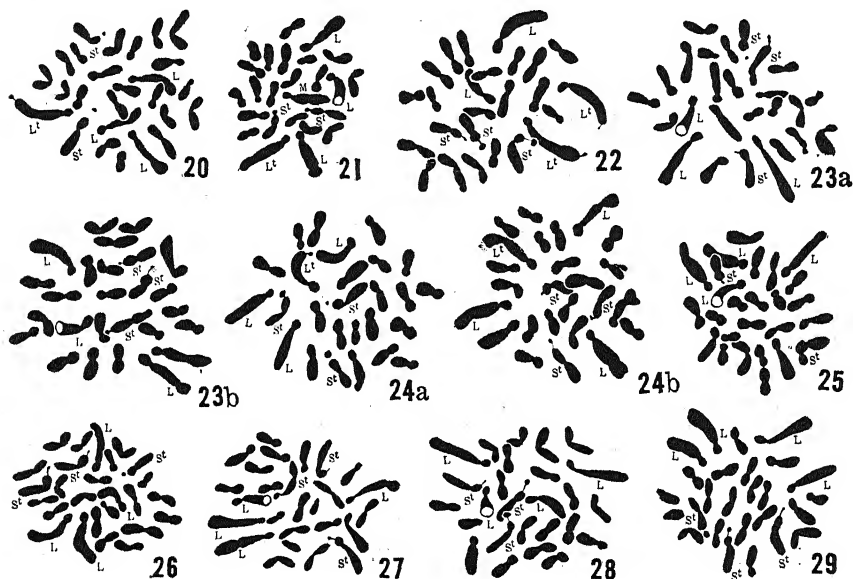
$$2n = 25 = 1L_1^+ + 1L_1 + 2L_2 + 1M + 2S^t + 18S \quad (\text{fig. 21})$$

*T. hirta* has six SAT-chromosomes ( $2L_1^+ + L_2^+ + 3S^t$ ) and *T. formosana* var. *stolonifera* has four SAT-chromosomes ( $2L_1^+ + 2S^t$ ) and their hybrids have only three SAT-chromosomes, namely one long chromosome having a small satellite at the distal end ( $L_1$ ) and two short chromosomes having large satellites at their proximal ends. One hybrid (fig. 21) has 25 somatic chromosomes of which one comparatively large chromosome (M) seems to be derived from the 25-chromosomal individual of *T. formosana* var. *stolonifera*, while another hybrid has 26 somatic chromosomes (fig. 20). These hybrids ought to have more than three satellites, but they have really only three and show disappearance of the satellite or differential amphiplasty. The long chromosomes with satellites belonging to *T. hirta* ( $2L_1^+ + L_2^+$ ) definitely disappeared in these hybrids.

2) *T. hirta* × *T. formosana*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$   
(fig. 23)

$2n = 25 = 2L_1^t + 2L_2 + 3S^t + 18S$   
(fig. 22)

One individual has 25 somatic chromosomes of which two long chromosomes have satellites at their distal ends and three short chromosomes have satellites at their proximal ends. *T. hirta* has six SAT-chromosomes ( $2L_1^t + L_2^t + 3S^t$ ) and *T. formosana* has only one SAT-chromosome ( $S^t$ ), so five SAT-chromosomes found in their



Figs. 20-29. Interrasid, interspecific and intergeneric hybrids in *Tricyrtis* and *Brachycyrtis*. 20, *T. hirta* × *T. formosana* var. *stolonifera*  $2n = 26 = 1L_1^t + 1L_1 + 2L_2 + 2S^t + 20S$ . 21, *T. hirta* × *T. formosana* var. *stolonifera*  $2n = 25 = 1L_1^t + 1L_1 + 2L_2 + 1M + 2S^t + 18S$ . 22, *T. hirta* × *T. formosana*  $2n = 25 = 2L_1^t + 2L_2 + 3S^t + 18S$ . 23, *T. hirta* × *T. formosana*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ . 24, *T. hirta* × *T. hirta* f. *variegata*  $2n = 2L_1 + 1L_2^t + 1L_2 + 3S^t + 19S$ . 25, *T. hirta* × *T. formosana* var. *lasiocarpa*  $2n = 26 = 2L_1 + 2L_2 + 2S^t + 20S$ . 26, *T. hirta* × *Brachycyrtis macrantha*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ . 27, *T. hirta* × *T. yatabeana*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ . 28, *T. hirta* × *T. hirta* f. *albida*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ . 29, *T. hirta* f. *albida* × *T. hirta*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$ . ×1750.

hybrid suggest translocation of the satellite, besides elimination of one short chromosome (fig. 22). Another individual has 26 somatic chromosomes of which three short chromosomes have satellites at their proximal ends. This hybrid clearly indicates the disappearance of the satellite of the long chromosomes belonging to *T. hirta* (fig. 23).

- 3) *T. hirta* × *T. hirta* f. *variegata*  $2n = 26 = 2L_1 + 1L_2^s + 1L_2 + 3S^t + 19S$  (fig. 24)

*T. hirta* has six SAT-chromosomes ( $2L_1^s + L_2^s + 3S^t$ ) and *T. hirta* f. *variegata* has four SAT-chromosomes ( $2L_2^s + 2S^t$ ) while their hybrid has 26 somatic chromosomes of which one long chromosome ( $L_2^s$ ) has satellite at the distal end and three short chromosomes have satellites at their proximal ends (fig. 24). Whether this long SAT-chromosome is derived from *T. hirta* or *T. hirta* f. *variegata* has not been made clear, its derivation from the latter may be probable. In such case the satellites of the long chromosomes belonging to *T. hirta* must have definitely disappeared in the hybrid.

- 4) *T. hirta* × *T. formosana* var. *lasiocarpa*  $2n = 26 = 2L_1 + 2L_2 + 2S^t + 20S$  (fig. 25)

This hybrid has 26 somatic chromosomes of which two short chromosomes have now been found to have satellites at their proximal ends. This SAT-chromosome number has not, however, been determined exactly, for it has not so far been possible to observe an adequate number of favourable metaphase plates.

- 5) *T. hirta* × *B. macrantha*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$  (fig. 26)

*T. hirta* has six SAT-chromosomes ( $2L_1^s + L_2^s + 3S^t$ ) and *B. macrantha* has five SAT-chromosomes ( $2L_1^s + 3S^t$ ) and their hybrid has only three short chromosomes with satellites ( $3S^t$ ). This observation indicates a disappearance of the satellite of the long chromosomes.

- 6) *T. hirta* × *T. Yatabeana*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$  (fig. 27)

*T. hirta* has six SAT-chromosomes ( $2L_1^s + L_2^s + 3S^t$ ) and *T. Yatabeana* has two SAT-chromosomes ( $2S^t$ ) while their hybrid shows only three short SAT-chromosomes. Disappearance of the satellite was observable in the case of the long chromosomes belonging to the *T. hirta*.

- 7) *T. hirta* × *T. hirta* f. *albida*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$  (fig. 28)

*T. hirta* has six SAT-chromosomes ( $2L_1^s + L_2^s + 3S$ ) and *T. hirta* f. *albida* has three SAT-chromosomes ( $3S^t$ ) while their hybrid has 26 somatic chromosomes of which only three short chromosomes have satellites at their proximal ends ( $3S^t$ ). In this case disappearance of the satellite of the long chromosomes is also observed.

- 8) *T. hirta* f. *albida* × *T. hirta*  $2n = 26 = 2L_1 + 2L_2 + 3S^t + 19S$  (fig. 29)

This hybrid is a reciprocal hybrid of the type mentioned above and has the same chromosome complement. Three short SAT-chromosomes are found and two SAT-chromosomes have large satellites and while the remaining one has a small satellite. The disappearance of the satellite belonging to *T. hirta* is clearly detected as in the other cases of hybridization.

Table 2. Comparison of karyotypes of hybrids in *Tricyrtis* and *Brachycyrtis*

Hybrids (Plant no.)	Karyotypes (2n)	Nucleoli
<i>T. hirta</i> × <i>T. formosana</i> var. <i>stolonifera</i> No. 1 (14 = 4 × 9)	26 = 1L <sub>1</sub> <sup>t</sup> + 1L <sub>1</sub> + 2L <sub>2</sub> + 2S <sup>t</sup> + 20S	3
<i>T. hirta</i> × <i>T. formosana</i> var. <i>stolonifera</i> No. 2 (14 = 4 × 9)	25 = 1L <sub>1</sub> <sup>t</sup> + 1L <sub>1</sub> + 2L <sub>2</sub> + 1M + 2S <sup>t</sup> + 18S	3
<i>T. hirta</i> × <i>T. formosana</i> (26 = 4 × 13)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 3S <sup>t</sup> + 19S	3
<i>T. hirta</i> × <i>T. formosana</i> (15 = 4 × 13)	25 = 2L <sub>1</sub> <sup>t</sup> + 2L <sub>2</sub> + 3S <sup>t</sup> + 18S	5
<i>T. hirta</i> × <i>T. hirta</i> var. <i>variegata</i> (17 = 4 × 16)	26 = 2L <sub>1</sub> + 1L <sub>2</sub> <sup>t</sup> + 1L <sub>2</sub> + 3S <sup>t</sup> + 19S	4
<i>T. hirta</i> × <i>T. formosana</i> var. <i>lasiocarpa</i> (19 = 4 × 5)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 2S <sup>t</sup> + 20S	(2)
<i>T. hirta</i> × <i>B. macrantha</i> (20 = 4 × 7)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 3S <sup>t</sup> + 19S	3
<i>T. hirta</i> × <i>T. Yatabeana</i> (22 = 4 × 2)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 3S <sup>t</sup> + 19S	3
<i>T. hirta</i> × <i>T. hirta</i> f. <i>albida</i> (25 = 4 × 12)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 3S <sup>t</sup> + 19S	3
<i>T. hirta</i> f. <i>albida</i> × <i>T. hirta</i> (32 = 12 × 4)	26 = 2L <sub>1</sub> + 2L <sub>2</sub> + 3S <sup>t</sup> + 19S	3

#### (VI) Nucleolar condition in the pollen grain mitosis

The correspondence between SAT-chromosomes and nucleoli was ascertained in several species and hybrids of *Tricyrtis* and *Brachycyrtis*, but some species showed a contradiction of Heitz's hypothesis of SAT-chromosomes and have nucleolar chromosomes in addition to these SAT-chromosomes. Almost all hybrids showed differential amphiplasty or the disappearance of satellite of the parent, that is, there were only three short SAT-chromosomes. Long chromosomes with satellites were found only in some inter-specific hybrids between *T. hirta* × *T. formosana*, *T. hirta* × *T. formosana* var. *stolonifera* and intraspecific hybrids such as between *T. hirta* × *T. hirta* f. *variegata*, *T. hirta* × *T. hirta* f. *albida* and its reciprocal hybrids. Clearly the long chromosomes with satellites belonging to *T. hirta* (2L<sub>1</sub><sup>t</sup> + L<sup>t</sup>) disappeared even in these hybrids. To clarify such confusion of satellite behaviour in the progeny, the pollen grain mitoses were observed carefully.

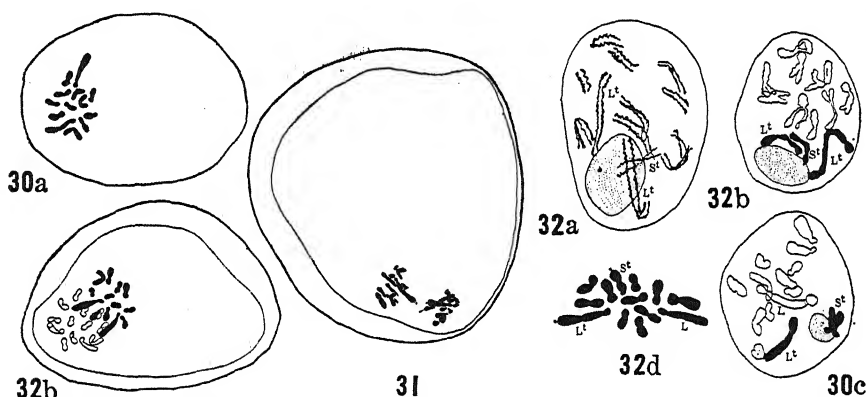
Two difficulties were, however, encountered in observing the pollen grain mitosis in *Tricyrtis*, that is, firstly the pollen grain sheath makes the necessary observation so difficult that, in order to see the chromosomes more easily, we have to peep into the pollen grain from its base where its patterns are very small, and secondly



the pollen grain mitosis has a definite position and direction in the narrow part of the pollen grain (figs. 30, 31), so we cannot easily follow the relation between chromosomes and nucleoli in the prophase stage.

1) *Tricyrtis formosana* ( $2n = 26$ ) (fig. 30)

This species has only one short SAT-chromosome and four nucleoli in the root-tip cell. Consequently we can rarely see the short SAT-chromosome in the pollen grain mitosis. Because of the difficulties mentioned above, the writer could not make a through observation in the prophase stage, but the relation between chromosomes and nucleoli seems to be the same as in the case of *T. perfoliata* which has four nucleoli and no SAT-chromosomes in its root-tip cell. Many chromosomes seem to become attached to the nucleoli, but neither the number or the type of the chromosomes can be determined. The nucleoli in the pollen grain number either one or two.



Figs. 30-31. The first division of pollen grains in *Tricyrtis*. Notice the position and direction of mitotic figures in the large raum of pollen grains. 30, *T. formosana*  $2n = 2L_1^t + 2L_2^t + 1S^t + 3S^n + 18S$ . a, polar view of pollen grain from its back. b, side view of pollen. 31. Side view of pollen grain in *T. formosana* var. *lasiocarpa*  $2n = 26 = 1L_1^t + 1L_1 + 2L_2^t + 1S^t + 21S$ .  $\times 1080$ . Fig. 32. Pollen grain mitosis in *T. formosana* var. *lasiocarpa*  $2n = 26 = 1L_1^t + 1L_1 + 2L_2^t + 1S^t + 21S$ . a-c, prophase,  $2L^t$ ,  $2L^t + 1S^t$  and  $1L^t + 1S^t$  attach to the nucleoli. d, metaphase,  $n = 13 = 1L^t + 1L + 1S^t + 10S$ .  $\times 1450$ .

2) *T. formosana* var. *lasiocarpa* ( $2n = 26$ ) (figs. 31, 32)

This species has four SAT-chromosomes i.e., three long and one short chromosomes and the corresponding four nucleoli in the root-tip cell. Consequently the SAT-chromosomes in pollen grain may be of the four types  $2L^t + 1S^t$ ,  $2L^t$ ,  $1L^t + 1S^t$  and  $1L^t$  in the same proportion, so the one, two and three SAT-chromosomes may be present in the proportion of 1:2:1. From the correspondence of SAT-chromosomes and nucleoli, we can expect that the numbers of

nucleoli in the pollen grain are one, two and three and that the ratio of these is, moreover, 1:2:1. In this expectation the number of nucleoli was counted in the resting stage and prophase of the pollen grain and one, two and three nucleoli were actually found, but the ratio of these numbers was contrary to expectations because of the fusion of nucleoli (cf. Tab. 3).

Table 3. Number of nucleoli in the pollen grains of *Tricyrtis formosana* var. *lasiocarpa*

Nos. of nucleoli per nucleus	1	2	3
Frequency	55	45	7

The SAT-chromosomes in the prophase become attached to the nucleoli and different combinations of SAT-chromosomes are also observed but the

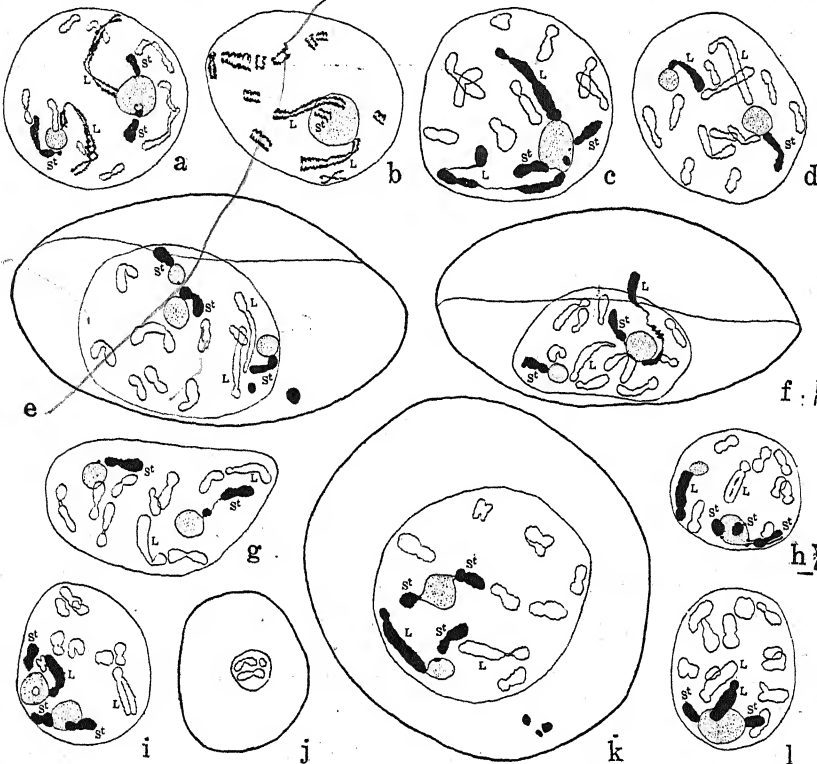


Fig. 33. Pollen grain mitosis in the hybrid ( $2n = 26$ ) between *T. hirta* and *T. formosana*. a,  $n = 13 = 2L^n + 3S^t + 8S$ . b,  $n = 13 = 2L^n + 1S^t + 10S$ . c,  $n = 13 = 2L^n + 2S^t + 9S$ . Notice the stretched long chromosome which perhaps made chromatid bridge in meiosis. d,  $n = 14 = 1L^n + 1L + 1S^t + 11S$ . e,  $n = 13 + 2ff = 2L + 3S^t + 8S + 2ff$ . f,  $n = 13 = 1L^n + 1L + 2S^t + 9S$ . Notice the stretched long chromosome which perhaps made chromatid bridge in meiosis. g,  $n = 12 = 2L + 2S^t + 8S$ . h,  $n = 12 = 1L^n + 1L + 3S^t + 7S$ . i,  $n = 13 = 1L^n + 1L + 3S^t + 8S$ . j. micro-nucleus with two chromosomes and one fragment. k,  $n = 12 + 3ff = 1L^n + 1L + 3S^t + 7S + 3ff$ . l,  $n = 15 = 1L^n + 1L + 2S^t + 11S$ .  $\times 1450$ .

ratio in each case could not be determined statistically on account of the paucity of favourable cases.

3) *T. hirta*  $\times$  *T. formosana* ( $2n = 26$ ) (fig. 33).

*T. hirta* has both long and short SAT-chromosomes and *T. formosana* has one short SAT-chromosome and long nucleolar chromosomes (in the strict sense), while their hybrid has only three SAT-chromosomes in the root-tip cell and definitely shows differential amphiplasty or disappearance of the satellites of the long chromosomes. We can easily imagine that this hybrid has two or three long SAT-chromosomes or nucleolar chromosomes and three short SAT-chromosomes, based upon the karyotypes of both parents.

The pollen grain mitosis was so carefully followed in the prophase, that the interesting fact was revealed that one or two long chromosomes become attached to the nucleoli, besides the short SAT-chromosomes. The nucleoli in the prophase of the pollen grain were found to be one, two, three and four in number. On account of the irregularities in meiosis the chromosomes also vary in number from eleven to sixteen (cf. Tab. 4). Bearing this in mind, we can imagine that the following combinations of nucleolar chromosomes probably exist in the pollen grains, namely  $2L^n + 3S^t$ ,  $2L^n + 2S^t$ ,  $2L^n + 1S^t$ ,  $1L^n + 3S^t$ ,  $1L^n + 2S^t$ ,  $1L^n + 1S^t$ ,  $3S^t$  and  $2S^t$ . Such cases mentioned above are really observed in

Table 4. Distribution of chromosomes in the pollen grains of hybrid ( $2n = 26$ ) between *T. hirta* and *T. formosana*

Nos. of chromosomes per nucleus	11	12	13	14	15	16
Frequency	3	16	17	12	2	1

Table 5. Number of nucleoli in the pollen grains of hybrid ( $2n = 26$ ) between *T. hirta* and *T. formosana*

Nos. of nucleoli per nucleus	1	2	3	4
Frequency				
Resting stage	16	34	17	4
Prophase	9	14	2	—
	25	48	19	4

Table 6. Number of nucleolar chromosomes in the pollen grains of hybrid ( $2n = 26$ ) between *T. hirta* and *T. formosana*

Nos. of nucleolar chromosomes per nucleus	1	2	3	4	5
Frequency	0	7	10	6	1

Table 7. Various combinations of nucleolar chromosomes in the pollen grains of hybrid ( $2n = 26$ ) between *T. hirta* and *T. formosana*

Combinations of nucleolar chromosomes	Frequency	Nos. of nucleolar chromosomes
$2L^n + 3S^t$	1	5
$2L^n + 2S^t$	2	4
$L^n + 3S^t$	4	4
$2L^n + S^t$	2	3
$L^n + 2S^t$	5	3
$3S^t$	3	3
$L^n + S^t$	3	2
$2S^t$	2	2

the prophase of the pollen grains (cf. Tab. 7), and such combinations as  $2L^n + 3S^t$ ,  $L^n + 3S^t$  and  $3S^t$  are derived from a lack of non-disjunction of the nucleolar chromosomes in meiosis, that is the distribution of homologous chromosomes in the same pole. Theoretically we can imagine such combinations as  $2L^n$ ,  $L^n$  and  $S^t$ , but these can not be found in the present case of observation.

Occasionally the long chromosomes attached to the nucleolus are identified as  $L_1$  with subterminal secondary constriction or as  $L_2$  with submedian secondary constriction. When two long nucleolar chromosomes become attached to nucleoli in the same cell, one chromosome (perhaps  $L_1$  of *T. hirta*) becomes attached at the proximal end and another one (perhaps  $L_2$  of *T. hirta* and  $L_1$  of *T. formosana*) becomes attached at the distal end.

## Discussion

### 1. Karyotype analysis in *Tricyrtis*

(a) **Chromosome number.** Almost all species belonging to this genus have 26 somatic chromosomes, only one hypoploid species being found in the course of the present observation. Some plants of *Tricyrtis formosana* var. *stolonifera* have 25 chromosome ( $2n-1$ ) in the root-tips, while the other plants of this variety have 26 chromosomes as in other species. This hypoploid plant has twenty short and five long chromosomes of which one extra-long chromosome may have resulted from fusion or translocation of two short chromosomes. Such a karyotype alteration may be transmitted in the progeny, so some hybrids between *T. hirta* and *T. formosana* var. *stolonifera* have 25 chromosomes with such an extra-long chromosome, while the other plants have normal chromosome complements. Another example of hypoploid ( $2n-1$ ) was found in a hybrid between *T. hirta* and *T. formosana*. This hybrid has also 25 chromosomes, suggesting elimination of one short chromosome. This hypoploid seems to have arisen as a result of its paternal parent having one heterozygous pair of short chromosomes. This pair shows inversion or translocation and often form a chromatid bridge in meiosis and consequently pollen grains with twelve chromosomes may result. The hypoploid may be produced as a result of hybridization between a normal egg cell with 13 chromosomes and such a pollen grain with 12 chromosomes.

The karyotype alterations such as fusion, translocation or inversion in parental species suggest possibility of many aneuploid plants being produced in their progeny. These irregularities in meiosis of both parent and hybrid will be reported in detail in another paper.

(b) **Chromosome size.** The chromosome in *Tricyrtis* shows variations in both length and breadth between the different species. Such variation may be detected by comparison between the karyotypes of *T. formosana* var. *ramosa* and *T. perfoliata* (fig. 34). The former species has a long chromosome complement with six satellites, while the latter has a short chromosome complement without satellites.

*T. Yatabeana* has a complement with two satellites, the chromosomes being shorter than in other species except *T. perfoliata* which has no satellite. This phenomenon suggests the "Chromosomenverkürzung" (Delaunay 1926) in *Tricyrtis*, that is, the chromosomes become shorter, the satellites become smaller (cf. Satô 1937 in Aloinae) and disappear. The variation of chromosome size is detected in

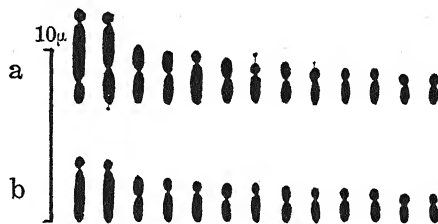


Fig. 34. Comparison of the karyotypes of *T. formosana* var. *ramosa* and *T. perfoliata* with basic chromosome sets. a, *T. formosana* var. *ramosa*. b, *T. perfoliata*.  $\times 2250$ .

different root-tips in the same individual i.e., large root-tips tend to have large chromosome complements and small root-tips tend to have small ones. In the same root-tip the dermatogen cells have large chromosome complements, while periblem cells have comparatively large ones and the pith cells small ones. Sometimes, however, the dermatogen cells are long, but too narrow, so that the metaphase plates also show a narrow arrangement of chromosomes. The periblem cells are, in all cases, the most suitable subject for comparison of chromosome sizes.

(c) **Karyotype.** The *Tricyrtis* plants have 26 somatic chromosomes except for one species and two hybrids and the chromosome size varies in different species, but generally speaking the chromosome complements are similar to each other so long as consideration of the SAT-chromosomes is excluded. When we take into consideration the SAT-chromosomes, various karyotypes are found even in the same species (fig. 35). Asymmetry of the SAT-chromosomes is clearly shown in many species and in all hybrids formed between them. In these cases, translocation of the satellites is plainly observable, but neither translocation of chromosome segments including a satellite nor translocation of satellites alone can be determined, unless the chromosome behaviour in meiosis is observed. Even when we cannot distinguish the difference between two karyotypes, the chromosome behaviour of hybrid between them in meiosis may suggest a karyotype alteration such as inversion or translocation.

In the present observation the original karyotype in *Tricyrtis* can not be determined by reason of the complexity of the various SAT-chromosomes, but six-SAT-chromosomes types (two long and

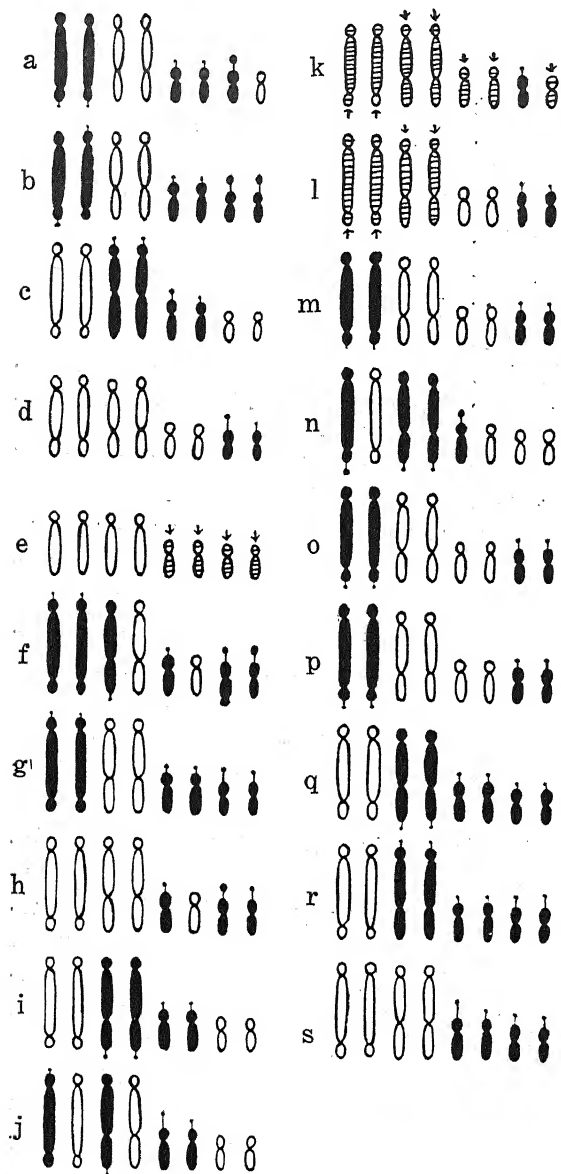


Fig. 35. Comparison of various combinations of SAT-chromosomes and nucleolar chromosomes in *Brachycyrtis* and *Tricyrtis*. Chromosomes in black are SAT-chromosomes, those hatched are nucleolar chromosomes which attach to the nucleoli at their ends shown by an arrow, and those outlined are ordinary chromosomes usually free from the nucleoli and rest of 17 or 18 chromosomes are omitted in these diagram. a, *B. macrantha* (Japanese name: Tosano-zyôrôhototogisu). b, *B. macranthiopsis* (Kiino-zyôrôhototogisu). c, *T. latifolia* (Tamagawa-hototogisu). d, *T. Yatabeana* (Kibanano-hototogisu). e, *T. perfoliata* (Tukinuki-hototogisu). f, *T. hirta* No. 1 (Hototogisu). g, *T. hirta* No. 2 (Hototogisu). h, *T. hirta* f. *albida* (Siro-hototogisu). i, *T. hirta* f. *variegata* (Hui-ri-hototogisu). j, *T. hirta* f. *albo-marginata* (Hukurin-hototogisu). k, *T. formosana* (Taiwan-hototogisu). l, *T. formosana* var. *stolonifera* (Turu-hototogisu). m, *T. formosana* var. *stolonifera* (Turu-hototogisu). n, *T. formosana* var. *lasiocarpa* (Ke-hototogisu). o, *T. formosana* var. *amethystina* (Hirohataiwan-hototogisu). p, *T. formosana* var. *kotoensis* (Kôtô-hototogisu). q, *T. formosana* var. *ramosa* (Edauti-hototogisu). r, *T. affinis* (Yamazino-hototogisu). s, *T. dilatata* (Tyōsen-hototogitu).  $\times 2250$ .

four short SAT-chromosomes) may represent the original karyotype, though the position of the satellite in the long chromosomes remains undetermined for the present, either at the distal end or at the proximal end.

## 2. Relation between chromosome and nucleoli

It is an interesting fact that *Tricyrtis* species, though they have the same chromosome number, have various combinations of the nucleolar chromosomes and are characterized by different nucleolus-chromosome relationships.

According to Matsuura (1938) the nucleolar chromosomes may be divided into two types: 1) Interstitial type, in which the nucleoli develop at the constricted portion of the SAT-chromosomes, and 2) Terminal type, in which the nucleoli develop definitely at the distal ends of definite chromosomes.

The interstitial or SAT-chromosome type was reported by many recent investigators in several plants (cf. S. Navashin 1922; Sorokin 1924, 1929; Senjaninova 1926; Hollingshead 1930; Heitz 1931a, b; Sax 1932. Dermen 1933; Smith 1933; Beadle 1934; Francini 1934; Geitler 1934, 1936; Lorbeer 1934; Leven 1935, 1936; Richardson 1935 a, b; Lesley and Lesley 1935; Darlington 1935; Fernandes 1935, 1936, 1937; Matsuura 1935, 1938; Satô 1936, 1937a, b, c, 1938; Resende 1936, 1937a, b; Westergård 1936; Raghavan 1938; Sinotô 1938; Kuhn 1938; Gates and Pathak 1938; etc.) and animals (cf. Heitz 1933; Heitz und Bauer 1933; Kaufmann 1934, 1937; Dearing 1934; Chen 1936; Patau 1937; Kawaguchi 1938).

In contrast with the interstitial type, several papers have hitherto dealt with the terminal type which Gates (1938) defined as a terminal satellite without a thread, that is, Darlington (1932) in *Agapanthus umbellatus* and *Kniphofia aloides*, Goodspeed (1934) in *Nicotiana longiflora*, Lorbeer (1934) in *Frullania dilatata*, Matsuura (1935, 1938) in *Trillium kamtschaticum*, Fernandes (1936) in *Narcissus bulbocodium* var. *genuinus*, Geitler (1936) in *Cladophora alpina*, Upcott (1936) in *Eremurus spectabilis*, Nandi (1937), Sakai (1938) and Parthasarathy (1938) in *Oryza sativa*, Bhatia (1938) in *Triticum dicoccum* and Tanaka (1938) in *Scirpus lacustris*. Sinotô (1937) found only one SAT-chromosome in  $F_1$  plants between two different strains of *Rumex acetosa*, but there were two nucleoli in the prophase. No other instances such as the co-existence of SAT-chromosome and nucleolar chromosome without satellite or a secondary constriction have been reported apart from the cases observed by McClintock and Sinotô with special reference to nucleolus formation, though such disappearance of satellites is a common occurrence in several plants (cf. Navashin (1934) in *Crepis* hybrids, McClintock (1934) in *Zea mays*, Ono (1935) and Yamamoto (1938) in *Rumex acetosa*, Satô (1937) in *Aloinae* and Satô (1937) in *Tricyrtis*).

Matsuura (1938) assumed two principles with regard to the nucleolar chromosome i.e., first, every chromosome can be referred to as a "nucleolar chromosome" in the sense that it can produce nucleoli under certain particular circumstances, and secondly, there is usually a differential rate in the capacity for nucleolus-organizing activity of chromosomes within a complement, which thus results in the usual occurrence that particular chromosomes alone are apparently related to the formation of nucleoli. Based on these two principles, the formation of small nucleoli or nucleolus-like bodies in *Vicia* (cf. Heitz 1931b), *Zea* (cf. McClintock 1934, Creighton) and *Trillium* (cf. Matsuura 1938), can be explained when SAT-chromosomes are absent.

It was shown from the present observation that both two pairs of long and short chromosomes in *Tricyrtis* have a greater rate of functional activity for nucleolus formation at their chromosome ends than elsewhere. This may be expressed by saying that the valency of these nucleolus-forming region is 2 or 3 and that of the remaining parts of the chromosomes is lower than 2. When in the case of such nucleolar chromosomes there is translocation of their terminal ends with greater valencies with respect to each other or to the other chromosomes or *vice versa*, new chromosomes with greater valencies such as 3, 4, 5 and 6 are formed and the presence of satellites at their ends may be indicated by subsequent nucleolus formation. Bearing such a possibility in mind, we can imagine that the SAT-chromosomes in *Tricyrtis* have various valencies (3, 4, 5 and 6) and normally the chromosomes with the greater valencies utilized the available material for production of a nucleolus in advance so that the chromosomes with lower valencies have little or no opportunity to function. However, under special conditions this competition might be disturbed, particularly when the difference in valency is small. This will result in the mobilisation of supernumeral nucleolar chromosomes in *Tricyrtis formosana*, *T. formosana* var. *stolonifera* and *T. perfoliata*, as in *Trillium kamtschaticum* where C-chromosome is occasionally attached to a nucleolus (cf. Matsuura 1938) and *Narcissus reflexus* where some chromosomes are occasionally attached to a nucleolus at the secondary constriction (cf. Fernandes 1936). And such a consideration will be also applicable to the cases of supernumeral nucleoli reported by Heitz (1931a) in *Allium* and *Aloe*, by Satô (1937a) in *Aloe*, by Resende (1937) in some Aloinae plants and by Matsuura (1938) in *Trillium*.

Heitz (1931a) noticed that when two SAT-chromosomes were present in a nucleus, distinctive size difference of the nucleoli can



result (cf. Resende 1937; Satô 1937) and the same reference may be possible in the case of many nucleolar chromosomes in *Tricyrtis perfoliata* (cf. Matsuura 1938 in *Trillium*). These observations support the conception of differential functional abilities of nucleolar chromosomes.

That the nucleolus in the telophase originates in the agglomeration of small nucleolus-like globules has been noticed by several authors (cf. Van Camp (1924) in *Clivia*; Dermen (1933) in *Callisia* and *Paeonia*; Mantón (1935) in *Biscutella*; Nandi (1937) in *Oryza*). "In the heterotypic telophase the nucleolar substance present in each chromosome appears in the form of small globules which are organized into a definite body, the nucleolus" (cf. Nandi 1937), while the nucleolus also originates in the form of small globules at the satellite stalk or secondary constriction in the telophase (cf. Heitz 1931b in *Vicia*, Gates and Pathak 1938 in *Crocus*). Suita (unpublished) observed in the telophase of the pollen tube division in the living material of *Crinum latifolia* ( $2n = 22 = 2L + 10M + 2M^* + 8S$ ) that one small globule-like vacuole appears symmetrically in each daughter nucleus and that each nucleolus was then organized from this globule.

In abnormal cases many nucleolus-like bodies are formed right at the chromosome end and subsequently dispersed over the whole body of the cell without later fusion into a large nucleolus in the telophase in *Zea mays* (cf. McClintock 1934; Creighton) and *Fritillaria* (cf. Frankel 1937).

All these evidences support the validity of the two principles concerning the nucleolar chromosome (cf. Matsuura 1938) mentioned above and indicate the necessity for a revision of Heitz's theory of the SAT-chromosome to reconcile it with the recent observations of the nucleolar chromosomes. It may therefore be concluded that nucleolar material present in each chromosome becomes collected as small nucleolus-like globules on the surface (especially at the chromosome ends, primary and secondary constrictions) of each chromosome and is therefore organized into a definite number of nucleoli by the agency of the nucleolar bodies in *Tricyrtis* and *Brachycyrtis*. When the difference of nucleolus-forming capacities of the nucleolar chromosomes is small, many other chromosomes may become attached to the nucleoli, even though many such chromosomes cannot condense respectively to a definite nucleolus.

As regards the general relation between the nucleolus and chromosomes, micro-chemical studies, such as those of Zucharias (1882), Yamaha and Sinotô (1925), Zirkle (1928, 1931) and Marshak (1931) have established the fact that there is an intimate resemblance be-

tween the substance of the nucleolus and the matrix of the chromosomes. Many recent investigators (cf. Dermen (1933), McClintock (1934), Matsuura (1935, 1938), Nandi (1937), Raghavan (1938) and Gates (1938) etc.) have suggested that the nucleolar substance contributes to the matrix of the chromosomes. Raghavan (1938) observed in *Polanisia trachysperma* that a portion of the chromatin contained in the chromosomes is found to have accumulated on the surface of the primary nucleolus, whose surface consequently shows darkly stained patches from the late telophase up to the late prophase. The close association of the prochromosomes with the nucleolus at the telophase and early prophase is, moreover explained on this basis.

In most cases the satellites consist of heterochromatin and can be clearly observed on the surface of the nucleoli in the telophase and resting stage, although small ones are invisible in *Tricyrtis*. Existence of the same condition may be recalled in the case of the nucleolar bodies which, as emphasized by Gates' school, may be clearly distinguished in *Zea* (cf. McClintock 1934), *Narcissus* (cf. Fernandes 1936) and *Oryza* (cf. Nandi 1937), while they may be invisible in *Trillium* (cf. Matsuura 1938). The former type means the terminal satellite without a thread, as defined by Gates (1938). The satellite stalk and secondary constriction are of chromosome origin and contain thimo-nucleic acid, being stained by Feulgen's nucleal method (cf. Fernandes (1937) in *Narcissus*, *Aloe* and *Vicia*; Gates and Pathak (1938) in *Crocus*).

### 3. Karyotype alteration and mobilization of the nucleolar chromosome

The karyotype alterations, especially translocation, inversion and elimination of SAT-chromosomes and satellites bring on a new balance in the nucleolus-chromosome relationship and sometimes the mobilization of the nucleolar chromosomes. The hybridization which implies the combination of two chromosome sets (or sometimes genomes) also established such a new balance and may result in differential amphiphasy i.e., the disappearance of satellites or contrarily in the mobilization of the nucleolar chromosomes. The pollen grains of the heterozygous plants and hybrids have various combinations of the chromosomes and consequently imply a new balance in the nucleolus-chromosome relationship and thus the new nucleolar chromosome member may be mobilized in the hybrid between *T. hirta* and *T. formosana* (fig. 33).

In *Scilla permixta* the translocation of a short SAT-chromosome or satellite results in formation of new chromosomes ( $M_3-S_3$ , or

s-M<sub>4</sub>) while the nucleolus-chromosome relationship also undergoes changes and consequently large and small nucleoli are formed in these combinations (cf. Satô 1936). Such a reference is also true in the case of *Nerine undulata* which has no typical secondary constriction and translocated segment (cf. Satô 1938). The pollen grains of such plants also have a new balance in the nucleolus-chromosome relationship and mobilization of the nucleolar chromosomes seems to occur (Satô unpublished). In *Scilla sibirica* no typical SAT-chromosome is found and the nucleolus is formed at the primary constriction of the E-chromosome (cf. Satô 1939a).

Such a relation between the nucleolus and the chromosome can be clearly explained on the basis of the differential rate in the capacity for nucleolus-forming activity of the chromosomes within a complement.

Many former observation on the relation between the nucleoli and the SAT-chromosomes (cf. Bushnell (1936) in Labiatae; Okuno (1937) in *Lobelia*; etc.) and recent observations on secondary constrictions which have no relation to nucleolus formation (cf. Fernandes 1935, 1936 a, b, 1937; Satô 1935, 1936, 1937, 1938; Heitz 1935; Resende 1936, 1937 a, b; etc.) will have to be reinvestigated in the light of the conception of "nucleolar chromosomes".

### Summary

1) Various karyotypes of two species belonging to *Brachycyrtis* and seven species, six varieties and three races belonging to *Tricyrtis* have been analysed from the view point of karyotype alteration. These plants all have 26 somatic chromosomes ( $2n = 26 = 4L + 22S$ ) except *T. formosana* var. *stolonifera* ( $2n = 25$ ) and two hybrids between *T. hirta*  $\times$  *T. formosana* and *T. hirta*  $\times$  *T. formosana* var. *stolonifera* (cf. Tab. 1, 2). The chromosome size in *Tricyrtis* varies between the different species as regards both length and breadth and it is an interesting fact that as the chromosomes in a complement become shorter the satellites become smaller and disappear (cf. fig. 34).

2) Various combinations of the nucleolar chromosomes (including the SAT-chromosomes) are found in these species and their hybrids. Homozygous and heterozygous types of SAT-chromosomes in *Tricyrtis* show the validity of Heitz's theory which assumes a correspondence between SAT-chromosomes and nucleoli at the telophase, while some plants of *T. formosana* var. *stolonifera* ( $2n = 26 = 2L_1^{\text{I}} + 2L_2^{\text{I}} + 2S^{\text{I}} + 20S$ ) have only two short SAT-chromosomes and four nucleoli in the telophase and *T. formosana* ( $2n = 26 = 2L_1^{\text{I}} + 2L_2^{\text{I}}$

+  $S^t$  +  $3S^n$  +  $18S$ ) has only one short SAT-chromosome and one large and three small nucleoli in the telophase while *T. perfoliata* ( $2n = 26 = 4L + 4S^n + 18S$ ) has no SAT-chromosomes and four nucleoli in the telophase. In these cases many nucleolar chromosomes, besides the SAT-chromosomes, become attached to the nucleoli and this phenomenon namely mobilization of the nucleolar chromosomes may be explained on the basis of Matsuura's principle concerning the nucleolar chromosomes which assumes that first, every chromosome can be referred to as a "nucleolar chromosome" in the sense that it can produce nucleolus under certain specified conditions, and secondly, there is usually a differential rate in the capacity for nucleolus-organizing activity of the chromosomes within a complement.

3) Many hybrids between these plants the SAT-chromosomes and the nucleoli correspond in number, but the satellites of long chromosomes with small satellites from *T. hirta* have disappeared in these hybrid in most cases. Such disappearance of the satellite or differential amphiplasty is clearly observed in *Tricyrtis* hybrids as in *Crepis* (cf. Navashin 1934).

4) The pollen grain mitosis in *T. formosana*, *T. formosana* var. *lasiocarpa* and in hybrids between *T. hirta* and *T. formosana* ( $2n = 26$ ) was observed and, in addition to SAT-chromosomes, nucleolar chromosomes were found in the case of the hybrid i.e., long chromosomes as well as short SAT-chromosomes became attached to the nucleoli (cf. Tab. 7).

The disappearance of the satellite in hybrids, and mobilization of the nucleolar chromosomes in the root-tips and pollen grains are explained on the basis of the conception of "nucleolar chromosomes".

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# Experimentelle Untersuchungen lebender Zellen in der Teilung. IV. Die Einwirkung des Dampfgemisches von Ammonia und Chloroform auf die Mitose bei den *Tradescantia*-Haarzellen<sup>1)</sup>

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(Mit 40 Textfiguren)

*Eingegangen am 25. Dezember 1938*

Wie ich schon in früheren Arbeiten erwähnt habe, weisen Dämpfe des Ammoniaks und des Chloroforms keine qualitative Veränderung der Spindelsubstanzen auf, während sie auf die Teilungsfigur von *Tradescantia*-Haarzellen reversibel einwirken (WADA 1937, 1938). Läßt man jedoch diese Dämpfe als Dampfgemisch auf die Teilungsfigur einwirken, so erweist sich das Dampfgemisch gegen die Teilungsfigur ganz anders als das Verhalten der Dämpfe im Einzelnen. Es ruft dabei verschiedene Störungen des Spindelmechanismus und auch dadurch das Zustandekommen verschiedener Teilungsanomalien im lebenden Zustande der Zelle hervor.

In vorliegender Arbeit habe ich mich bemüht, zunächst die Störungen des Spindelmechanismus und verschiedene Teilungsanomalien zu untersuchen, welche unter der Einwirkung des Dampfgemisches Ammonia-Chloroform zustande kommen. Weiterhin werden auf Grundlage dieser Experimente Beziehungen zwischen den spindelbildenden und den phragmoplastbildenden Substanzen, der normale und abnormale Zytokinesemechanismus und die Einzelwesenheit der Spindelfigur erörtert.

## Methode

Das Dampfgemisch von Ammonia und Chloroform wird durch folgende drei Methoden hergestellt. 1. Die zwei Waschflaschen, von denen die eine Ammoniak und die andere Chloroform enthält, werden, zwischen der Mundpipette und dem Doppelgebläse durch Gummiröhren miteinander verbunden, eingeschaltet. Durch das Drücken des Doppelgebläses leitet man die Dämpfe von den Flaschen in die Mundpipette, wobei sich die beiden Dämpfe miteinander vermischen. 2. Man mischt einen Teil Ammoniak mit 20–30 Teilen

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1) Contributions from the Divisions of Genetics and of Plant-Morphology, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 233.



Chloroform in einem Scheidetrichter und schüttelt das Gemisch einige Male. Dann benutzt man die untere Schicht als den Ursprung des Dampfgemisches. 3. Man mischt eine kleine Menge Chloroform mit einer großen Menge 0.01-proz. Ammoniak und schüttelt das Gemisch in einem Scheidetrichter viele Male. Aus diesem Gemisch nimmt man die obere Schicht als den Ursprung des Dampfgemisches.

In Bezug auf das Mengenverhältnis von Ammonia zu Chloroform wurde aus Erfahrung festgestellt, daß die Wirkung des Dampfgemisches auf die Spindelfigur höchst effektiv ist, wenn der Chloroformdampf eine nur geringe Menge von Ammoniadampf enthält<sup>1)</sup>. Aus dem Dampfgemisch, welches durch die Methode 2 hergestellt und längere Zeit im Vorrat gehalten wurde, emanierte der Chloroformdampf reichlicher als der Ammoniadampf, während aus dem durch die Methode 1 oder 3 hergestellten alten Dampfgemisch der Ammoniadampf reichlicher emanierte. Bei solchen alten Dampfgemischen prägt sich oft die Einwirkung des Ammoniadampfes allein oder die des Chloroforms allein auf die Teilungsfigur aus. Es ist also zu empfehlen, das Dampfgemisch vor dem Gebrauch frisch herzustellen.

Als Untersuchungsmaterial benutzte ich junges isoliertes Staubfadenhaar von *Tradescantia reflexa*, welches eine sich in der Teilung befindende Zelle enthielt. Die Lebendbeobachtung, die Behandlung der Teilungsfigur mit dem Dampfgemisch, die Kultivierung der behandelten Zellen und die Mikrophotographierung wurden fast in gleicher Weise ausgeführt, wie in früheren Arbeiten. Daher wird eine eingehende Beschreibung darüber hier weggelassen. Alle Figuren im Text sind in ca. 900-facher Vergrößerung reproduziert.

### Beobachtung

Die Anzahl der behandelten Zellen in der Teilung betrug 90, von denen 19 während der Behandlung abstarben. 32 Zellen wiesen Störungen der Spindelfiguren auf und bildeten dadurch verschiedene Teilungsanomalien: nämlich Syndiploidkerne, Zwergkerne, kernlose

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1) Die Entstehung der zwei Syndiploidkerne, welche ich (1938 S. 99) beim Versuche der Dampfwirkung des Chloroforms auf die Teilungsfigur von *Tradescantia*-Haarzellen erhielt, läßt sich wie folgt erklären: Um die Teilungsfiguren dem Chloroformdampf auszusetzen, wandte ich dieselbe Gummiröhre an, welche bei Ammoniadampf-Versuchen gebraucht wurde. Dadurch emanierte eine geringe Menge des Ammoniadampfes aus der Gummiröhre und vermischte sich mit dem Chloroformdampf. Also wirkten die Dämpfe am Anfang der Experimente zweimal als Dampfgemisch von Chloroform und Ammonia auf die Spindelfigur ein und bildeten Syndiploidkerne. Bei weiteren Versuchen emanierte aber der Ammoniadampf nicht mehr aus der Gummiröhre und es traten dabei keine Teilungsanomalien im lebenden Zustande auf.

oder zweikernige Zellen und andere komplizierte abnorme Teilungsfiguren. Weitere 34 Zellen führten ihre Kern- und Zellteilung in normaler Weise zu Ende, einige von ihnen zeigten jedoch ungleich große Tochterkerne oder gekrümmte Querwände. Die restlichen 5 Zellen in der Prophase kehrten nach der Behandlung zum Ruhezustande zurück.

Tabelle 1. Die durch Behandlung mit dem Dampfgemisch Ammonia-Chloroform hervorgerufenen Teilungsbilder

Teilungsstadien am Nach d. Anfang der Ex- Behandlung perimente entstandene Teilungsbilder	Prophase			Meta- kine- se	Meta- phase	Anaphase			Telophase			Summe	
	I	II	III			I	II	III	I	II	III	lebend	koaguli- ert
Teilungsumkehr													
lebend	1	4										5	
koaguliert			1										1
Riesenkern													
mit od. ohne Wandreste				1	3	2	7	1					
mit Zwergkern u. Wändchen					2	1	3						
mit kernlosem Raum					2		1	1				24	
koaguliert						2		2					4
zwei- od. mehrkernige Zelle													
mit od. ohne Wandreste								4	1				
mehr als 3 räumig							3					8	
koaguliert							1						1
Bildung d. Tochterkerne u. -zellen													
normal		1		4	3	9	7	4		1	1		
mit ungleich groß. Kernen od. abnorm. Scheidewand							2	2				34	
koaguliert			1	1	2	2	4	1		2			13
Summe													
lebend			6	5	10			47			3	71	
koaguliert			2	1	2			12			2		19

Bemerkung: I, II und III bedeuten resp. frühe, mittlere und späte Zeit einzelner Teilungsphasen.

**Zytoplasma:** Das Dampfgemisch Ammonia-Chloroform übt auf das Zytoplasma einmal eine überwiegende Wirkung von Chloroformdampf, und ein anderes Mal solche von Ammoniadampf aus.

Im ersten Fall prägt sich die Entmischung des Zytoplasmas aus und im letzteren Fall die Quellung des Zytoplasmas und die Vakuolenkontraktion (Fig. 1–2, 3–4). Jedoch entmischt sich späterhin das angequollene Zytoplasma nach und nach (WADA 1937, 1938).

Bei Zufuhr der geeigneten Menge des Dampfgemisches auf die Teilungsfigur sind die oben angegebenen Veränderungen des Zytoplasmas fast immer reversibel, daher können sich die behandelten Zellen nach der Entfernung der Dampfwirkung zum gesunden Zustande erholen. Ihre Teilungsvorgänge gehen dabei den Umständen gemäß normalerweise oder abnormalerweise vor sich.

**Spindelfigur oder Atraktosom:** Die Einwirkung des Dampfgemisches Ammonia-Chloroform prägt sich besonders in der Spindelfigur oder im Atraktosom aus. Die Benennung „atractosome“ ist von FUJII vorgeschlagen und wurde letzthin von YASUI (1939) in ihrer Abhandlung angeführt. Das Atraktosom bedeutet das Atraktoplasma als einen Körper und wir verstehen unter Atraktoplasma eine mit Zytoplasma nicht mischbare, an Wasser mangelnde, granulene, flüssige Substanz, welche zusammen mit Spindelfasern und Chromosomen die Einzelwesenheit der Spindelfigur beibehält (FUJII 1931, WADA 1932, 1935 u. a.). Unter der Wirkung des Dampfgemisches quellen zuerst die Chromosomen im Spindelraum. Da einzelne Gestalten der Chromosomen durch ihre Quellung unsichtbar sind, sieht das Atraktosom homogen aus. Während dieser Veränderung quellen oder verflüssigen nicht nur das Atraktoplasma, sondern auch die Spindelfasern.

Infolge der Quellung der Spindelsubstanzen oder Vergrößerung ihrer Fluidität rundet die Spindelfigur zuerst ihre Pole ab, und wird bald danach ellipsenförmig (Fig. 6, 9, 21, 25, 32). Dies beweist, daß das Atraktoplasma und die Spindelfasern infolge ihrer Fluiditätszunahme ihren Oberflächenraum zu vermindern suchen. Sie nehmen zusammen mit angequollenen Chromosomen als Ganzes eine homogene ellipse Form an. Bei diesem Zustande der Spindelfigur ist besonders bemerkenswert, daß diese homogene ellipsenförmige Masse vollkommen frei von Granulen und durch eine Grenzfläche vom Zytoplasma geschieden ist. Dieses Verhalten der Spindelfigur beweist auch, daß das Atraktosom selbst im angequollenen Zustande mit dem Zytoplasma nicht mischbar ist.

Das Atraktosom wird auch in der normalen intakten Teilungsfigur der *Tradescantia*-Haarzellen als ein Einzelwesen anerkannt. Man stößt jedoch bei intakten Spindeln auf die Schwierigkeit, ihre Grenzfläche zu verfolgen, da die Grenzfläche von dem Atraktosom und dem Zytoplasma nicht immer glatt ist, wie wir uns ihrem

Namen nach vorstellen. Die Durchsichtigkeit des Atraktoplasmas, das Angefülltsein der Chromosomen und die Zytoplasmaarchitektur um das Atraktosom herum, lassen auch die Einzelwesenheit des Atraktosoms immer schwer erkennen.

Unter der Wirkung des Dampfgemisches verliert die angequollene Spindel die Fähigkeit die Chromosomen in die Spindelpole zu bringen, ungeachtet, ob die Chromosomen ihre Längsspaltung ausgeführt haben oder nicht. Sie bleiben daher in der Kernplatte, aber ihre Anordnung wird gestört. Der angequollene Zustand der Spindelfigur dauert nicht lange; sie fängt bald als Ganzes zu entquellen an. Zuerst treten die durch die Quellung unsichtbar gewordenen Chromosomen wieder im Spindelraum in Erscheinung. Weitere Veränderungen des Atraktosoms weisen auf, daß sich das angequollene Atraktoplasma unmittelbar an der Entstehung der phragmoplast-bildenden Substanz und an der Bildung der Querwand beteiligt. Einige Beispiele abnormaler Kern- und Zellteilungen habe ich im nächsten Abschnitte ausführlich beschrieben.

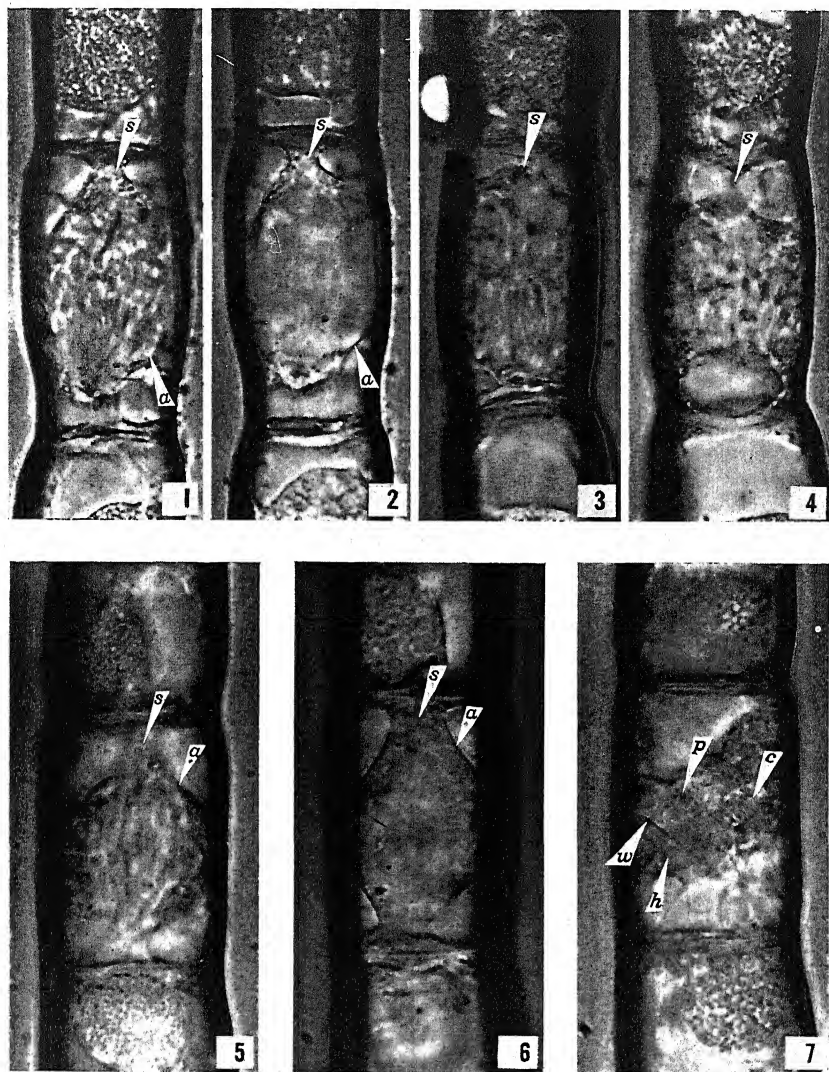
**Chromosomen:** Bei der Einwirkung des Dampfgemisches besteht kein Zweifel, daß die Quellung der Chromosomen und auch das Verschwinden ihrer einzelnen Gestalten auf der Wirkung des Ammoniadampfes beruhen. Während des angequollenen Zustandes der Spindelfigur geht die Entquellung der angequollenen Chromosomen allmählich vor sich. Durch die Entquellung erreichen die angequollenen Chromosomen nicht nur den normalen, sondern weiter den entwässerten Zustand und die Chromonematisierung einzelner Chromosomen wird schließlich ausgeprägt. Auffallend ist auch, daß die Chromosomen nach Verflüssigung der Spindelfasern eine Neigung haben, vom frei gewordenen Zustande sich zu in einen Klumpen zusammenzuballen. Schließlich verwandeln sich die chromonematisierten Chromosomen zu einem oder einigen ruhenden Kernen verschiedener Größe.

Während dieser Veränderungen der Chromosomen entstehen verschiedene Teilungsanomalien, die teils der Teilungsphase der behandelten Zellen, teils der Intensität der Dampfwirkung entsprechen.

Von den verschiedenen Teilungsanomalien gebe ich hier zunächst ein Beispiel über das Zustandekommen einer kernlosen Zelle zu

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nach der Behandlung. Neben dem Chromosomenklumpen, der sich durch Chromonematisierung zu einem Syndiploidkern verwandelt, tritt die phragmoplast-bildende Substanz als ein gesonderter, hyaliner Raum auf. In der Mitte des hyalinen Raumes entsteht eine Wandanlage und um den Raum herum häuft sich bewegliches, granulenreiches Zytoplasma an. *s* Spindelpol, *w* Wandanlage, *h* phragmoplast-bildende Substanz, *p* bewegliches granulenreiches Zytoplasma, *a* Grenzfläche des Atraktosoms, *c* Chromosomenklumpen.



Vergleiche der Dampfwirkung von Ammonia, Chloroform und des Dampfgemisches Ammonia-Chloroform.

Fig. 1-2. Einwirkung des Ammoniadampfes. 1. Vor der Behandlung. Mittlere Anaphase. 2. 2 Minuten nach der Behandlung. Durch Quellung der Chromosomen sieht das Atraktosom homogen aus, aber es behält sowohl die Teilungsfähigkeit als auch dieselbe Größe und Gestalt bei. Fig. 3-4. Einwirkung des Chloroformdampfes. 3. Vor der Behandlung. Frühe Anaphase. 4. 3 Minuten nach der Behandlung. Die Vakuolen vergrößern sich durch Zytoplasmaentmischung und beklemmen dadurch die Spindelfigur. Aber die Teilungsfähigkeit des Atraktosoms bleibt bestehen. Fig. 5-7. Einwirkung des Dampfgemisches Ammonia-Chloroform. 5. Vor der Behandlung. Mittlere Anaphase. 6. 1 Minute nach der Behandlung. Durch Quellung der Chromosomen und der Spindelsubstanzen sieht das Atraktosom homogen aus und vergrößert sich besonders an seinen beiden Spindelpolen. In diesem Zustande tritt eine Störung des Spindelmechanismus ein. 7. 36 Minuten

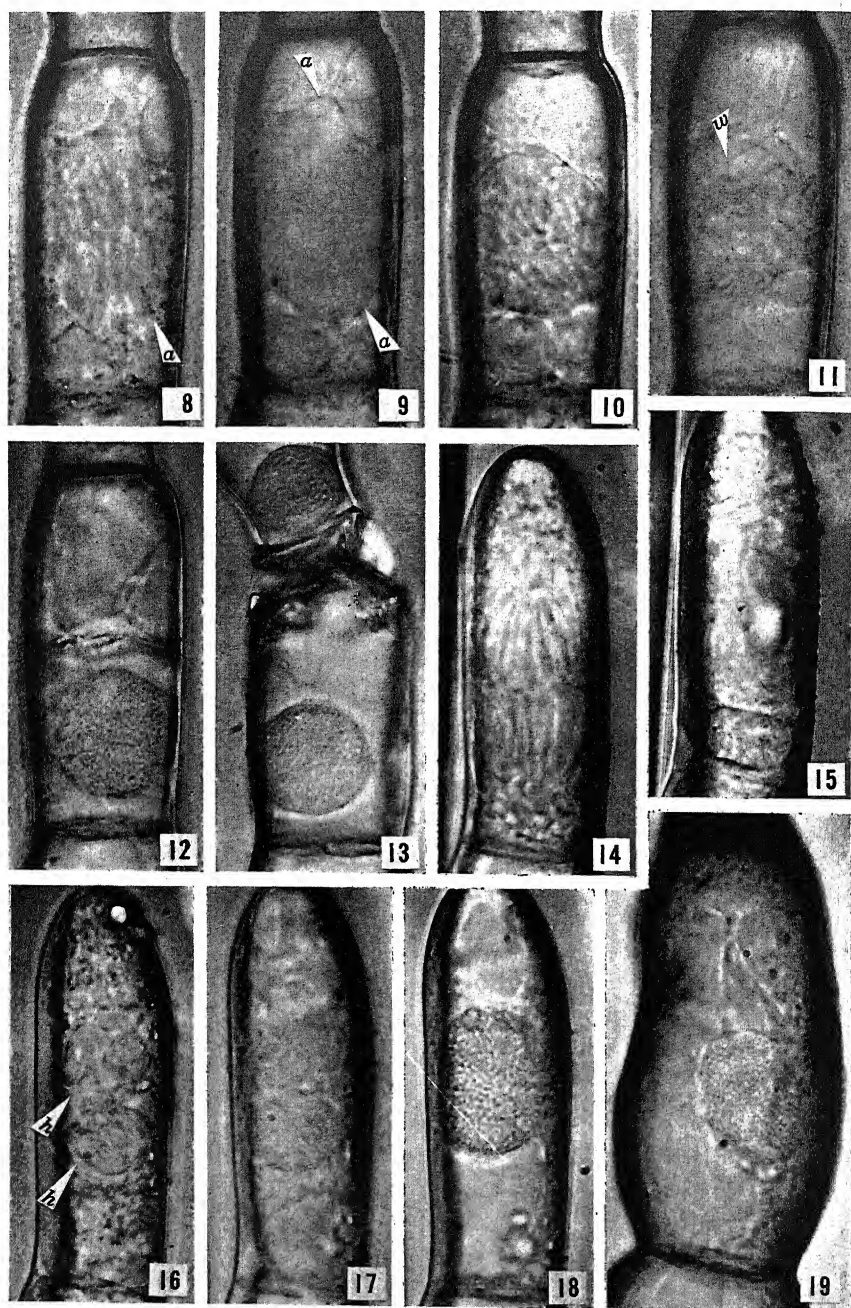


Fig. 8-13. Entstehung einer kernlosen Tochterzelle. Fig. 14-19. Verschmelzung der Tochterkerne. Ausführliche Erklärungen im Text. Siehe Seite 165ff und 167ff. *a* Grenzfläche des Atraktosoms, *w* Wandanlage, *h* phragmoplast-bildende Substanz.

Protokoll. Die behandelte Teilungsfigur befand sich in der fünften Zelle vom Haarende. Das Dampfgemisch wurde durch die Methode 1 hergestellt und den Zellen mit einer Mundpipette zugeführt. Der Innendurchmesser der Pipette betrug  $54\ \mu$ .

- 15:22<sup>1)</sup> am 16. Juni 1936. Frühe Anaphase. Vor der Behandlung (Fig. 8).
- 15:26. Die Teilungsfigur ist dem Dampfgemisch ca. 5 Minuten lang ausgesetzt. Eine Minute nach der Behandlung treten kleine Entmischungsvakuolen in der Nähe der Polplasmastränge auf. Die Spindelpole fangen schon an sich abzurunden.
- 15:29. Sowohl die Chromosomen als auch die Spindelsubstanzen quellen an; daher sieht die Spindelfigur als Ganzes homogen und ellipsenförmig aus (Fig. 9).
- 15:31. Aufhören der Dampfzufuhr.
- 15:33. Sowohl die Spindelfigur als auch das Zytoplasma bleiben noch anquellend. Jedoch ist die erstere ganz frei von Granulen, und unterscheidet sich vom letzteren durch Strahlenbrechen an ihrer Grenzfläche.
- 15:42. Jetzt tritt die Einwirkung des Ammoniadampfes auffallend ein. Um die angequollene Spindelfigur herum prägt sich die Quellung des Zytoplasmas und daher die Vakuolenkontraktion aus. In den Vakuolen treten kleine flüssige Füllungen auf und setzen die BROWNSche Bewegung fort. Im Gegensatz zum Verhalten des Zytoplasmas fängt die Spindelfigur als Ganzes zu entquellen an. Ebenfalls entquellen die Chromosomen und werden wieder sichtbar. Sie zeigen Neigung, sich ohne Wanderung zu Spindelpolen zu einem Klumpen zu vereinigen (Fig. 10).
- 16:14. Während der Chromonematisierung der Chromosomen häufen sich die angequollenen Spindelsubstanzen in der Nähe des Chromosomenklumpens an (oben in der Fig.) und bilden einen klaren hyalinen Raum (Fig. 11). Um diesen hyalinen Raum herum sammelt sich weiter flüssiges granulenreiches Zytoplasma, und die Granulen in ihm setzen lebhaft die BROWNSche Bewegung fort. An der äußersten Oberfläche dieser Zytoplasmaanhäufung machen sich amoeboiden Bewegungen des Protoplasmas und Entwicklung der Plasmastränge bemerkbar, die sich meist radial durch Vakuolen zur Mutterzellwand ausdehnen<sup>2)</sup>. Sobald sich der hyaline Raum

1) 15:22 bedeutet 15 Uhr 22 Minuten; die Worte Uhr und Minute sind (wie auch in den folgenden Zeitangaben) weggelassen.

2) BECKER (1933 S. 140) bemerkte auch in einer kernlosen Zelle und in seiner sogenannten Pseudoamitose eine fortdauernde Bewegung des Zytoplasmas und die Bildung einer abnormen Zellplatte. Diese Erscheinungen, die ich in dieser Arbeit durch verschiedene Beispiele ausführlich erklärt habe, scheinen nicht immer auf Degenerationsfällen der degenerierenden Phragmoplasten zu beruhen (freilich sind die Veränderungen bei der Vitalfärbung von BECKER nichts anderes als Degenerationsfälle der Teilungsfigur). Sogar bei normalen Zytokinesevorgängen kann man bei genauer Beobachtung eine bewegliche Zytoplasmamasse in der Umgebung vom Phragmoplasten wahrnehmen. Bei einer kernlosen Zelle oder bei anderen abnormen Mitosen ist aber die fortdauernde Bewegung des Zytoplasmas oder seine amoeboiden Bewegungen durch folgende Umstände deutlich sichtbar: Erstens benötigen bei abnormen Mitosen sowohl die Ausbildung der Querwand als auch die Wiederherstellung des Zytoplasmas zum gesunden Zustande längere Zeit. Daher dauert die Plasmabewegung vor und nach der Zellplattenbildung auffallend lange. Zweitens kommen die Zytoplasmaanhäufungen um die hyaline Substanz unmittelbar mit Vakuolen in Berührung und dehnen sich während der Zytokinesevorgänge in ihnen aus. Daher wird die Bewegung des Zytoplasmas dort auffallend erleichtert und anschaulich erweitert.



zu einer gewissen Größe entwickelt hat, tritt eine Wandanlage schnell in seiner Mitte auf, und wächst langsam weiter in dem sich immer vergrößernden hyalinen Raum.

Während dieses Zeitverlaufes bildet sich die Zelle als Ganzes auffallend wieder zum gesunden Zustande zurück. Der Chromosomenklumpen bildet jetzt einen ruhenden Riesenkern oder einen Syndiploidkern. Die Wandanlage streckt sich zusammen mit der hyalinen Substanz etwas schief nach rechts und links aus und formt endlich eine Zellplatte. Eine Scheidewand entsteht fast an normaler Stelle und in normaler Gestalt. Während dieser zytokinesischen Vorgänge spielt die hyaline Substanz die Rolle eines Phragmoplasten, formiert jedoch schließlich den sogenannten Phragmoplasten nicht.

17. Juni. Ein Tag nach der Behandlung. Die Mutterzelle hat sich jetzt in eine kernlose und in eine kernhaltige Tochterzelle geteilt. Die kernlose Zelle sieht schwach aus und es mangelt bei ihr an Granulen im Zytoplasma, jedoch zeigt sie noch hier und da Protoplasmaströmungen. In der kernhaltigen Zelle sind der Kern und das Zytoplasma vollständig gesund, indem das granulenreiche Zytoplasma lebhaft Plasmaströmungen aufweist (Fig. 12).
18. Juni. Die kernlose Zelle wird allmählich altersschwach und zeigt nur lokalisierte Plasmaströmungen. Außerdem ist die Scheidewand infolge der Abnahme des Turgordruckes von der Seite der kernhaltigen Zelle gedrückt und sinkt leicht ein. Die kernhaltige Zelle dagegen ist noch ebenso gesund wie gestern.
19. Juni. Die kernlose Zelle allein stirbt ab und verliert den Turgordruck gänzlich; daher ist sie jetzt infolge des Turgordruckes der nächst liegenden Zellen und auch durch ihr Wachsen gequetscht. Die kernhaltige Zelle wächst auch, bleibt aber gesund (Fig. 13).
24. Juni. Die kernhaltige Zelle zeigt jetzt Anzeichen der Altersschwäche; die Anzahl der Granulen im Zytoplasma nimmt beträchtlich ab.
27. Juni. Die Altersschwäche der Zelle schreitet weiter fort; die Kernstruktur verschwindet und sieht homogen aus, aber einige Nukleolen bleiben noch übrig. Die gewöhnlichen Granulen im Zytoplasma verschwinden gänzlich, unzählige winzige Granulen, welche lebhaft Bewegungen zeigen, treten im Zytoplasma auf.
30. Juni. Die kernhaltige Zelle stirbt auch infolge Altersschwäche ab.

Wie ich oben beschrieben habe, teilt sich eine Zelle mittels phragmoplast-bildender Substanz oder des Phragmoplasten zu einer kernlosen und einer kernhaltigen Tochterzelle. Nach gänzlichem Verbrauch der Ernährung in der Zelle wird die kernlose Zelle sofort altersschwach und stirbt ab. Ist hingegen die Scheidewand einer kernlosen Zelle nicht vollständig ausgebildet, und es sind durch irgend eine Perforation oder durch Risse plasmatische Verbindungen zwischen der kernlosen und der kernhaltigen Zelle übriggeblieben, so kann die kernlose Zelle oder auch der kernlose Raum als ein Teil der kernhaltigen Zelle mit ihr zusammen leben (Fig. 34, 37). Dem Zustandekommen einer vollständigen kernlosen Zelle wie in Fig. 8-13, begegnet man nur selten; häufiger scheiden sich die Teilungsfiguren durch Behandlung mit dem Dampfgemisch Ammonia-Chloroform zu einer Zelle mit einem Riesenkern und einer mit einem Zwergkern.



In Fig. 21 quillt die Spindelfigur unter der Wirkung des Dampfgemisches an und sieht wie ein homogener Raum aus. Die Zusammenballung der chromonematisierten Chromosomen zu einem Klumpen wird durch das Eindringen der angequollenen Spindelsubstanzen teilweise verhindert (Fig. 22). Die Scheidewandbildung geht dabei fast an normaler Stelle vor sich; daher enthält eine Tochterzelle einen Zwergkern und die andere einen Riesenkern (Fig. 23). In einem anderen Fall bildet zuerst die angequollene Spindel einen ellipsenförmigen hyalinen Raum (Fig. 25). Dann teilen sich die chromonematisierten Chromosomen durch Anhäufung der angequollenen Spindelsubstanzen zu einer großen und einer kleinen Masse, wobei die kleine durch die phragmoplast-bildende Substanz umgeben und von einer anderen Chromosomenmasse vollständig getrennt ist (Fig. 26). Daher treten Querwände um den kleinen Sonderkern herum auf und die Mutterzelle teilt sich zu einem kleinen Räumchen mit einem Sonderkern und zu einem großen Raum mit einem Riesenkern (Fig. 27).

**Verschmelzung der Tochterkerne:** Eine andere interessante Erscheinung ist, daß Schwesterkerne in einer durch Einwirkung des Dampfgemisches hervorgerufenen zweikernigen Zelle zu einem Syndiploidkern verschmelzen können. Eine derartige Kernverschmelzung beobachtete ich aber nur zweimal unter 32 verschiedenen Teilungsanomalien im lebenden Zustande; doch konnte ich die Verschmelzungsvorgänge durch Lebendbeobachtung ausführlich verfolgen. Hier gebe ich ein Beispiel zu Protokoll: Das Dampfgemisch wurde nach Methode 1 hergestellt und die Zellen wurden ihm mit einer Mundpipette ausgesetzt. Der Innendurchmesser der Pipette betrug  $54 \mu$ . Die Teilungsfigur befand sich in einer Terminalzelle eines Haares.

11:00 am 29. Juni 1936. Späte Anaphase. Vor der Behandlung (Fig. 14).

11:01. Das Haar ist zwei und eine halbe Minute lang dem Dampfgemisch ausgesetzt. Während zwei Minuten nach der Dampfzufuhr treten fast keine Veränderungen auf, dann aber gehen sie schnell vor sich. Zuerst sieht die Spindelfigur dünn und durch die Quellung der Chromosomen homogen aus, wobei die Quellung des Atraktosoms ausgeprägt wird. Während dieser Veränderungen quillt das Zytoplasma auch an, und nach und nach kontrahieren sich Vakuolen.

11:05. In den kontrahierten Vakuolen tritt eine Anzahl von flüssigen Fällungen auf. Diese Veränderungen beweisen, daß der Ammoniadampf jetzt stärker auf das Zytoplasma einwirkt als der Chloroformdampf (Fig. 15).

11:16. Das Zytoplasma fängt jetzt an, zu entquellen und sich zu erholen. Zytoplasmastränge dehnen sich von der sich erholten Zytoplasmamasse aus; die Tochterchromosomen sind im Begriff an jedem Spindelpol einen Tochterkern zu bilden. Um die Tochterkerne herum entstehen granulenhofe freie hyaline Höfe, um die sich granulenhofe flüssige Zytoplasmamassen häufen, und die Granulen in ihnen zeigen eine lebhaft bewegte Bewegung (Fig.

- 16). An Hand der Entstehungszeit dieser hyalinen Höfe und ihrer Lage besteht kein Zweifel, daß sie aus einer phragmoplast-bildenden Substanz bestehen. Da jede einzelne Anhäufung der Phragmoplast-Substanz in der Mitte einen Tochterkern enthält, ist es ihnen unmöglich eine Wandanlage in der Mitte des mit der Phragmoplast-Substanz erfüllten Raumes zu bilden (Siehe S. 170). Dieses eigenartige Verhältnis der Phragmoplast-Substanz zu den Tochterkernen ist der Grund dieses besonderen Falles, daß die Schwesterkerne in einer zweikernigen Zelle zu einem Syndiploidkern verschmelzen können.
- 12:04. In jedem Spindelpol liegt ein homogen aussehender, noch leicht angequollener, kugelförmiger Tochterkern; die Granulen im Zytoplasma vermindern sich beträchtlich und das Zytoplasma selbst sieht hyalinartig aus.
- 15:25. In den Tochterkernen prägt sich eine Chromonema-Struktur aus, wobei eine Anzahl von flüssigen Fällungen in hyalinen Zonen um die Tochterkerne auftreten. Diese Fällungen zeigen jedoch keine Bewegung. Das Verhalten der Tochterkerne macht jetzt den Eindruck, als ob sie ohne Bildung der Scheidewand eine zweikernige Zelle seien. In der Tat verschmelzen sich aber die Tochterkerne in der Nacht zu einem Syndiploidkern.
30. Juni. Die Zelle enthält bloß einen vollständig verschmolzenen Syndiploidkern, in welchem sich einige Nukleolen befinden. Das Zytoplasma hat sich noch nicht vollständig erholt, es ist granulenarm und eher altersschwach (Fig. 18).
1. Juli. Die Zelle stellt sich im Ganzen wieder zum gesunden Zustand her, und zeigt lebhaftes Protoplasmaströmungen. Die Struktur des Kernes ist ebenfalls ganz normal. Die Nukleolen in ihm sind daher schwer erkennbar.
4. Juli. Der Syndiploidkern, das Zytoplasma und die Protoplasmaströmungen sind normal und gesund. Am Terminalende der Zelle wird ein Teil des Zytoplasmas gelblich. Daß sich ein Teil des Zytoplasmas oder der Zellsaft zunächst gelblich und später bräunlich färben, ist keine besondere Erscheinung, die unter der Einwirkung des Dampfgemisches Ammonia-Chloroform zustande kommt. Diese Erscheinung findet auch sonst bei isolierten farblosen jungen Haaren von *Tradescantia* statt, wenn sie im hängenden Tropfen in einer Feuchtkammer längere Zeit kultiviert werden. Die Färbung des Zytoplasmas tritt gewöhnlich am Terminalende des Haares, an der Stelle der Scheidewand oder an der angestochene Stelle am Zytoplasmakoagulum auf, wobei sich das Zytoplasma zuerst gelblich, dann bräunlich färbt. Ebenso färbt sich das Zytoplasmakoagulum gelblich oder bräunlich, aber in seltenem Fall rötlich. Der Zellsaft wird gelblich, aber seine Färbung tritt immer später ein als beim Zytoplasma. Bei Kulturversuchen der isolierten Staubfadenhaarzellen von *Tradescantia* in der Feuchtkammer konnten wir nicht die Entstehung der violetten Farbe, welche die erwachsenen Staubfadenhaarzellen in der Natur produzieren können, feststellen.
8. Juli. Die Farbe des Zytoplasmas im Terminalende der Zelle wird allmählich bräunlich. Der Zellsaft färbt sich jetzt auch gelblich, aber die Zelle befindet sich als Ganzes noch im gesunden Zustande und zeigt viele lebhaftes Protoplasmaströmungen. Während dieser Kultur ist das Wachstum der Zelle auffallend (Fig. 19).
10. Juli. Die Zelle ist noch gesund. Beim Austausch der Mediumlösung aus der Feuchtkammer ging das Haar leider zufällig verloren.

In bezug auf experimentelle Untersuchungen der Mitose von *Tradescantia*-Haarzellen habe ich bereits festgestellt, daß zweiker-

nige Zellen, die unter Einwirkung von Anstichen, Plasmolyse und Austrocknung hervorgerufen werden, die Fähigkeit verlieren, durch Berührung der beiden Tochterkerne zu einem Syndiploidkern zu verschmelzen (WADA 1932 S. 123, u. a.).

Abweichend von früheren Ergebnissen ergibt das Dampfgemisch Ammonia-Chloroform in bezug auf die Verschmelzung der somatischen Kerne ein eigenartiges Resultat. NĚMEC (1910 S. 429) untersuchte das Zustandekommen der vegetativen Kernverschmelzungen experimentell durch verschiedene Eingriffe und stellte folgende zwei Bedingungen: die eine bezieht sich auf die Eigenschaft der Kernmembran und die andere auf die Berührung der beiden Kerne.

Wie ich oben bereits beschrieben habe, sind die Tochterkerne in der Telophase von hyalinen Höfen umgeben und kommen nicht mit dem Zytoplasma in Berührung. Unter diesen Umständen erscheint die Eigenschaft der Tochterkernoberfläche oder Tochterkernmembran stark abweichend vom normalen telophasischen Kern, während die Verschmelzungsfähigkeit der Tochterkerne längere Zeit beibehalten wird. Kommen solche fusionsfähigen Tochterkerne zufällig miteinander in Berührung, so findet ihre Verschmelzung nur in der hyalinen Substanz statt.

Daß sich die phragmoplast-bildende Substanz nicht zwischen den Tochterkernen, sondern um sie herum anhäuft und sowohl von der Bildung einer Wandanlage als auch von ihrer Plasmatisierung längere Zeit zurückgehalten wird, ist ein ganz ungewöhnliches Ereignis. Deshalb geschieht das Zustandekommen der Kernverschmelzung in zweikernigen Zellen nur selten.

**Phragmoplast und Mechanik der Querwandbildung:** Es ist besonders bemerkenswert, daß das Dampfgemisch Ammonia-Chloroform auf die Entwicklung der phragmoplast-bildenden Substanz und weiter auf die Querwandbildung wenig einwirkt, während es bei der Tätigkeit der Spindelfigur deutliche Störungen hervorruft.

Wie ich schon in den vorhergehenden Aufsätzen beschrieben habe, häufen sich die angequollenen Spindelsubstanzen nach der Störung der Spindeltätigkeit unregelmäßig neben der Chromosomenmasse an und bilden dort klar hyaline Räume. Die Zytoplasmaarchitektur, welche sich um die Spindelfigur herum befand, pflegt sich nunmehr um die hyalinen Räume herum anzuhäufen (Fig. 7, 11, 22, 29). Hinsichtlich des Wesens der Substanz im hyalinen Raum liefern die Ergebnisse der Lebendbeobachtungen dieser Experimente den Beweis dafür, daß die Substanz in ihm aus den angequollenen Spindelsubstanzen abgeleitet wird und nichts anderes als eine phragmoplast-bildende Substanz ist. Wenn der Raum durch

Anhäufung der hyalinen Substanz eine gewisse Größe erreicht hat, tritt eine Wandanlage schnell durch die Mitte des Raumes auf, entwickelt sich weiter zu einer Zellplatte und endlich zu einer Scheidewand.

Aus Lebendbeobachtung der 66 Teilungsfiguren bei diesen Experimenten konnte ich hinsichtlich des Zustandekommens der Querwand folgende zwei Bedingungen feststellen: 1. Das Vorhandensein der phragmoplast-bildenden Substanz. Diese Substanz zeigt sich nämlich in der normalen Zytokinese als Phragmoplast und in der abnormalen als klar hyaline Substanz neben dem Chromosomenklumpen. 2. Die Mitwirkung des Zytoplasmas. Außer der Phragmoplast-Substanz beteiligt sich bei der Querwandbildung auch das Zytoplasma, welches sich um den hyalinen Raum der Phragmoplast-Substanz und im Wandbelag der Mutterzelle befindet.

Man kann vielleicht sagen, daß die phragmoplast-bildende Substanz für die Scheidewandbildung unentbehrlich ist, aber es ist auch erwiesen, daß der sogenannte Phragmoplast für die Scheidewandbildung nicht immer unbedingt notwendig ist, wenn die oben angeführten zwei Bedingungen erfüllt werden. Die Wandanlage tritt in normaler Weise in der Äquatorialebene des Phragmoplasten und in abnormaler durch die Mitte des hyalinen Raumes auf, und in diesem entsteht nur eine einzige Wandanlage. Treten mehr als zwei hyaline Räume von einander getrennt in einer behandelten Teilungsfigur auf, so werden wenigstens am Anfang des Auftretens mehr als zwei Wandanlagen formiert.

Vor dem Auftreten der Wandanlage bemerkten YASUI (1939) in Haarzellen von *Tradescantia* durch Lebendbeobachtung, und BECKER (1934) in denselben Materialien durch Vitalfärbung, die Entstehung der Granulen in der Äquatorialebene des Phragmoplasten. Unter Einwirkung des Dampfgemisches muß auch die Entstehung dieser Granulen im hyalinen Raum bei der Bildung der Wandanlage vorangehen. Es ist jedoch fast unmöglich, die Entwicklung der Wandanlage von Anfang an zu verfolgen, da die junge Anhäufung der phragmoplast-bildenden Substanz nach der Störung der Spindeltätigkeit mit beweglichen granulenhaften Zytoplasmamassen bedeckt ist, und man kann deshalb das Auftreten der Wandanlage oder die Anhäufung der Granulen in der Mitte der Phragmoplast-Substanz nicht immer beobachten.

Die Mitwirkung des Zytoplasmas teils zur Zellplattbildung und teils zur Verfestigung der Scheidewand erweist sich als notwendig. Nach dem Auftreten streckt sich die Wandanlage aber langsam und zentrifugal im hyalinen Raum aus, wobei sie sich, entsprechend der Ausbreitung der hyalinen Substanz und der sie umgebenden Zyto-

plasmaanhäufung ausdehnt. Endlich erreichen sowohl die Wandanlage als auch die hyaline Substanz die Mutterzellwand und es bildet sich eine Zellplatte.

Wenn eine Wandanlage durch das Vorhandensein einer Vakuole die Mutterzellwand nicht erreichen kann, wirft die Zytoplasmaanhäufung zuerst viele Protoplasmastränge durch die Vakuole zur Mutterzellwand um den hyalinen Raum herum. Dann breiten sich sowohl die Zytoplasmamasse als auch der hyaline Raum in ihr aus, wobei sich eine fortdauernde Bewegung der Zytoplasmamasse an ihren Rändern bemerkbar macht. Durch diese Ausbreitung erreicht die Wandanlage auch die Mutterzellwand, wobei sich die feste Querwand nicht zentrifugal, sondern von dem zuerst die Mutterzellwand erreichten Teil der Wandanlage aus zum anderen Teil hin entwickelt.

Nach der Entstehung der Wandanlage im Phragmoplasten oder im hyalinen Raum bei behandelten Zellen wird angenommen, daß irgend eine Substanz aus dem Plasmawandbelag der Mutterzellwand an der Wandanlage oder an der Zellplatte entlang auswandert und daß sich die weiche Wandanlage erst durch Berührung mit dieser Substanz zentripetal oder von einer Seite aus zu einer festen Querwand entwickeln kann. Die Wanderung des Protoplasmas an der Oberfläche der MLI (Middle Lamella Initial) entlang bemerkte bereits YASUI (1939) und stellte fest, daß dieses ausgewanderte Zytoplasma der Mutterzelle die der MLI nächst anliegende Plasmamembran gestaltet. Das Zytoplasma der Mutterzellwand bildet demnach die Plasmamembran der Tochterzellen. Diese Wanderung des Protoplasmas scheint mir an der Verfestigung der Wandanlage zur Dauerquerwand beteiligt zu sein.

Aus Ergebnissen verschiedener Entwicklungsstufen und auch aus der experimentellen Untersuchung der Scheidewandbildung bin ich zu dem Schluß gelangt, daß der Teil der Wandanlage, welcher zuerst mit der Zellmembran in Berührung kommt, zuerst als feste Dauerzellwand auftritt und daß sich nicht immer der andere, der zuerst im hyalinen Raum erscheint, zuerst zur festen Querwand verwandelt.

In der Tat kommt es nicht selten vor, daß eine Durchlochung oder eine Öffnung an der Scheidewand bei der Entwicklung der Zellplatte durch Verhinderung eines Vakuolenraumes verursacht wird. Dagegen sind nie Fälle beobachtet worden, in welchen die Entwicklung einer festen Querwand bloß an der Berührungsstelle mit der Mutterzellwand verhindert wird und ein Teil des festen Wandstückes sich frei von der Mutterzellwand im Innern des Zellumens isoliert.

Bei Lebendbeobachtungen können wir auch oft bemerken, daß die Stelle einer ausgebildeten Scheidewand mit derjenigen der Wandanlage nicht immer übereinstimmt. Diese Tatsache dient auch zum Beweis, daß die Zellplatte selbst keineswegs eine Art von festliegender Querwand ist. Eine feste Querwand wird von der Stelle an formiert, wo die Wandanlage zuerst mit der Mutterzellwand in Berührung kommt. Beispiele dafür kann ich in Fig. 11 und 26 veranschaulichen, wo die Stelle der Wandanlage mit derjenigen der Querwand bloß an ihrem linken Ende übereinstimmt, weil die Wandanlage dort zuerst die Mutterzellwand erreicht hat (Fig. 12, 27).

**Verschiedene abnorme Querwandbildungen:** Der hier eingeführten Schlußfolgerung gemäß ist es möglich, verschiedene abnorme und natürlich auch normale Zytokinesevorgänge folgerichtig zu erklären. In nachstehender Beschreibung führe ich daher einige Beispiele als Beweis dieser Schlußfolgerung an.

In Fig. 28 tritt eine Wandanlage in einem klar hyalinen Raum auf. Ein Teil der Wandanlage kommt mit der Mutterzellwand, ein anderer mit einer großen Vakuole in Berührung. Während der Chromonematisierung des Chromosomenklumpens vergrößert sich der hyaline Raum in der Vakuole (oben in Fig. 29), aber die Wandanlage entwickelt sich sehr wenig. Späterhin verschwinden die meisten Teile der Wandanlage; nur der mit der Mutterzellwand in Berührung gekommene Teil der Wandanlage wird fest und steht als Faltung der Innenoberfläche der Mutterzellwand schwach hervor (Fig. 30). Fig. 31 zeigt die obere Ebene der Zelle, wo sich ein Syndiploidkern und ringförmige Faltungen an der Mutterzellwand befinden.

In Fig. 38b häuft sich die phragmoplast-bildende Substanz fast in der Mitte der Zelle an. Bald gestaltet die Substanz dort einen hyalinen Raum, in welchem eine Wandanlage der Längsnachse der Zelle parallel auftritt. Die Zytoplasmamassen verteilen sich jedoch meistens um die Chromosomenmasse und weniger um den hyalinen Raum. Daher wird der hyaline Raum durch Vakuolen von den Mutterzellwänden ferngehalten. Fig. 39b zeigt weiteres Wachstum des hyalinen Raumes und auch der Wandanlage. Wegen Mangels an Zytoplasma um den hyalinen Raum, entwickelt sich die Wandanlage nicht zur Zellplatte, und die Wandanlage verschwindet endlich durch die Plasmatisierung der hyalinen Substanz gänzlich. In Fig. 40b wandert der durch das Dampfgemisch hervorgerufene Syndiploidkern ohne irgendwelche Verhinderung an die Stelle, wo sich früher die Wandanlage befand.

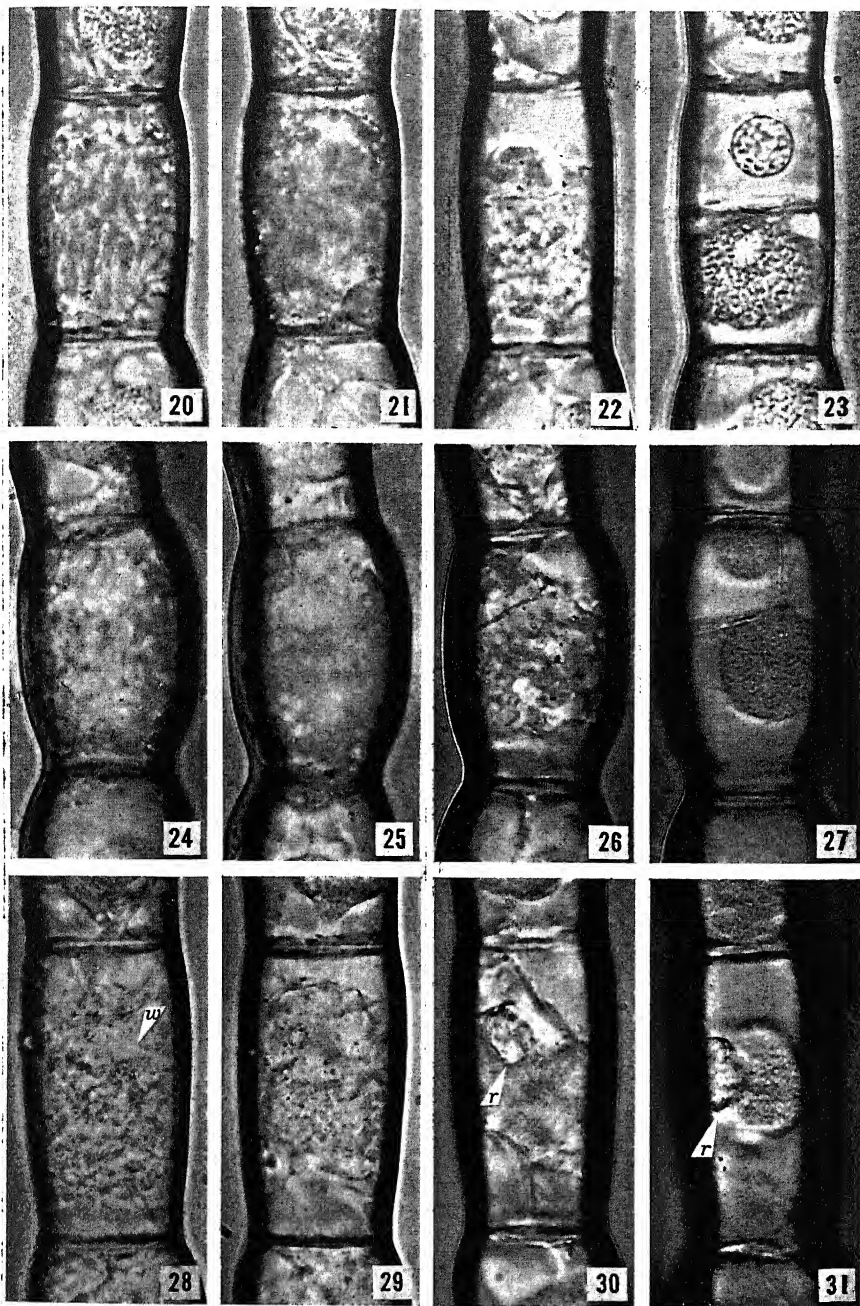


Fig. 20-23. Entstehung eines Zwerg- und eines Riesenkernes in der Tochterzelle. 20. 24. Juli 1936, 10:23. Behandlung 10:26 50 Sekunden. 21. 10:28. 22. 11:07. 23. 25. Juli. Fig. 24-27. Entstehung eines Sonderkernes mit einem kleinen Räumchen. 24. 2. Juni 1936, 10:29. Behandlung 10:33 4 Minuten. 25. 10:40. 26. 12:02. 27. 9. Juni. Fig. 28-31. Entstehung eines Syndiploidkernes mit einem Wandrest. 28. 28. Juli 1936, 9:51. Behandlung 9:16 25 Sekunden. 29. 10:20. 30. 13:10. 31. 29. Juli. Eingehende Erklärungen im Text. *w* Wandanlage, *r* Wandrest.



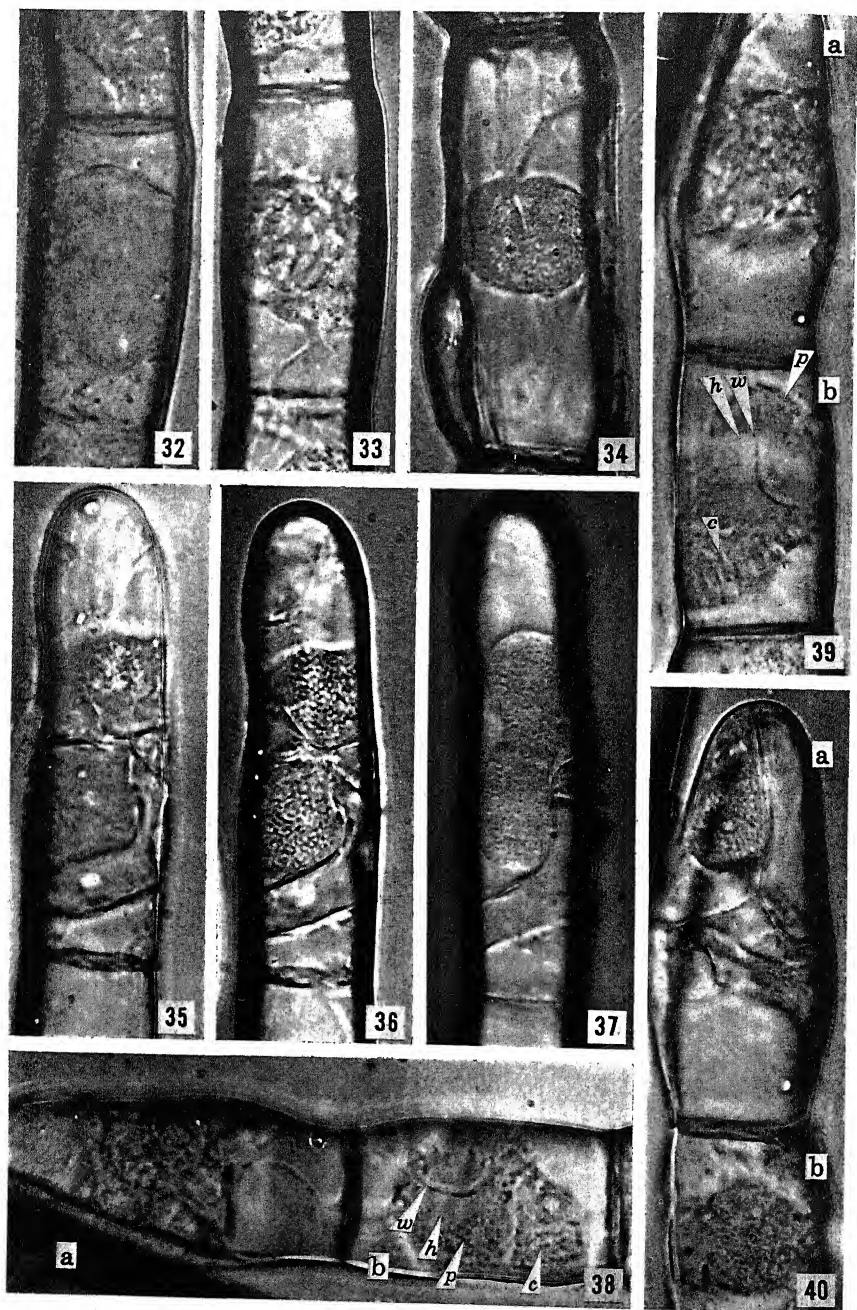


Fig. 32-34. Entstehung eines Syndiploidkernes und eines kernlosen Räumchens. 32. 24. Juli 1936, 10:59. Behandlung 10:58 45 Sekunden. 33. 11:24. 34. 31. August. Fig. 35-37. Ein teilweise geschnürter Riesenkern und komplizierte Querwände. Behandlung 20. Juli 1933, 11:54 50 Sekunden. Photos aufgenommen am 21. Juli. 35. unterer Teil der Zelle. 36. mittlere Ebene. 37. oberer Teil. Fig. 38-40. Ent-



Im Gegensatz zu diesem Beispiel trennen sich die chromonematisierten Chromosomen in Fig. 38a zu einigen unregelmäßigen Klumpen. Daher teilt sich die phragmoplast-bildende Substanz auch in einige Gruppen und bildet hier und da in der Nähe der Chromosomenklumpen kleine hyaline Räume. In Fig. 39a zeigt jeder der hyalinen Räume eine Wandanlage in sich und Wandanlagen, welche die oben erwähnten Bedingungen erfüllt haben, bilden ein festes Wändchen. Endlich hat sich die Mutterzelle in vier Räumchen mit drei Kernchen geteilt (Fig. 40a). Von diesen Räumchen ist das eine ein kernloses, aber sein Zytoplasma verbindet sich durch eine Lücke der Querwand mit demjenigen des kernhaltigen Räumchens. Daher kann der kernlose Teil der Zelle auch als ein Teil der kernhaltigen längere Zeit leben.

Fig. 35–37 zeigen eine sehr komplizierte Querwandbildung und einen Syndiploidkern, welcher eine sogenannte pseudoamitotische Figur darstellt. Fig. 35 zeigt den unteren Teil der Zelle, wo sich der größte Teil der phragmoplast-bildenden Substanz anhäuft, während sich die Chromosomen im oberen des Zellraumes befinden. Daher liegen komplizierte Wändchen hauptsächlich an der Innenseite der Grundfläche der Zelle. Von diesen Wändchen erweisen sich nur zwei als gekrümmte Querwände und die anderen als komplizierte Faltungen der Mutterzellwand. Fig. 36 zeigt die mittlere Ebene der Zelle. An dieser Stelle wurden die meisten Wändchen durch das Vorhandensein des Chromosomenklumpens an der Ausbildung vollständiger Querwände verhindert. Hier hat der Kern eine tiefe Schnürung und er sieht aus, als ob er durch das komplizierte Wändchen in zwei Teile geteilt wäre. In Fig. 37 erblickt man nur den oberen Teil der Zelle. Hier befinden sich ein Syndiploidkern, eine kurze schiefe und ein andere lange gekrümmte Querwand, welche einen Umweg um den Riesenkern macht.

Das Verhalten der phragmoplast-bildenden Substanz in Fig. 35, 36 und 40a beweist, daß sie Neigung hat, sich hauptsächlich in der Äquatorialebene der Zelle anzuhäufen; dort treten nämlich dicht in einem kleinen Raum Wändchen in Erscheinung. Weiter wurde festgestellt, daß eine kleine Wandanlage, welche sich sonst nie allein zu einer festen Querwand ausbilden kann, sich doch unter der Mitwirkung der am nächsten gebildeten Wandanlage und ihrer Zytoplasmaanhäufung zu einer festen Querwand entwickeln kann.

stehung einer mehrkernigen Zelle mit mehreren Räumchen und Verschwinden einer Wandanlage. 38. 3. Juli 1936, 11:00. Behandlung 10:28 20 Sekunden. 39. 11:13. 40. 6. Juli. Ausführliche Erklärungen im Text. *a* Grenzfläche des Atraktosoms, *h* phragmoplast-bildende Substanz, *c* Chromosomenklumpen, *w* Wandanlage, *p* bewegliches granulenreiches Zytoplasma.

## Schluß

Chemikalien, welche zum Auslösen verschiedener Teilungsanomalien verwendbar sind, wurden schon seit dem Anfang der Zellforschung von vielen Forschern gesucht. Man suchte zuerst den Einfluß verschiedener narkotischer Mittel und weiter den Einfluß von Säuren, Alkalien, Alkoholen, Alkaloiden und von verschiedenen anderen Stoffen zu erforschen. Neuerdings ist für den Zweck der zytologischen Förderung durch die Anwendung von Colchicin ein ausgezeichneter Erfolg erbracht worden; doch werden die Forscher Zeit und Mühe nicht scheuen, um noch effektivere und zugänglichere Stoffe zu finden.

Aus den Ergebnissen dieser Experimente sind wir jetzt hinsichtlich der Probleme des Mitosenmechanismus, der Chromosomenverdoppelung und anderer Zwecke, zu einer neuen Möglichkeit gelangt. Wir können nämlich durch Mischung geeigneter Chemikalien einen vortrefflichen Effekt auf die mitotischen Figuren erzielen, wenngleich die einzelnen Chemikalien auf die Mitose wenig einwirken. Daher ist es auch möglich, daß Chemikalien, welche sich einmal für das Auslösen der Chromosomenverdoppelung als wenig wirksam erwiesen haben, durch Anwendung geeigneter Partner als Gemisch effektiv wirken.

Bei den Einwirkungen des Dampfgemisches Ammonia-Chloroform handelt es sich um die Quellung der Spindelsubstanzen, welche weder durch Ammonia noch Chloroformdampf allein hervorgerufen werden können (WADA 1937, 1938). Über die Quellung der Spindelfigur und die dadurch hervorgerufenen Teilungsanomalien habe ich (1932, 1935) schon bei den Anstichversuchen der *Tradescantia*-Harzellen geschrieben und die Vermutung dabei geäußert, daß sich sowohl das Atraktoplasma als auch die Spindelfasern infolge der Aufnahme von überschüssigem Wasser von Außen durch die Stichstelle der Zellwand aufquellen oder sich verflüssigen. Diese Quellung der Spindelsubstanzen verursachen eine Störung des Spindelmechanismus und rufen dadurch verschiedene Teilungsanomalien im lebenden Zustande der Zelle hervor.

Unter der Einwirkung des Dampfgemisches kommt als Ursache der Quellung der Spindelfigur der Wasserverkehr in und außerhalb der Zelle kaum in Frage. Infolge der eigenartigen Einflüsse des Ammonia- und des Chloroformdampfes auf die Teilungsfigur bin ich zu folgender Schlußfolgerung gekommen: Unter der Einwirkung des Dampfgemisches quellen die Chromosomen zuerst durch den Ammoniadampf an; in diesem Zustande entmischen sich die Chromosomen durch Einwirkung des Chloroformdampfes und scheiden

ihr Quellungswasser, welches sonst schwer aus den Chromosomen zu den Spindelsubstanzen wandert, aus. Infolge dieses Befreiens der Chromosomen vom Quellungswasser kommen einerseits die Chromonematisierung der Chromosomen und andererseits die Quellung des Atraktoplasmas und der Spindelfasern zustande. Dadurch entsteht eine Störung der Spindeltätigkeit, wobei sich das angequollene Atraktoplasma und die Spindelfasern zur phragmoplastbildenden Substanz verwandeln und einen hyalinen Raum herstellen. Dann tritt immer eine Querwandbildung in diesem hyalinen Raum auf.

Wie ich durch Anstichversuche festgestellt habe, erbringen die Ergebnisse dieser Experimente auch den Beweis, daß sich die Spindelsubstanzen durch Quellung zur phragmoplastbildenden Substanz verwandeln. Neuerdings hat YASUI (1939) durch Lebendbeobachtung der Mitose von *Tradescantia*-Haarzellen festgestellt, daß der Phragmoplast, der seinen Ursprung im Atraktoplasma hat und sich bis zum Ende der Zytokinese von dem ihn umgebenden Zytoplasma unterscheidet, ein Derivat der Kernsubstanzen ist. Hinsichtlich des Ursprungs der phragmoplastbildenden Substanz und ihrer Einzelwesenheit bin ich mit YASUI gleicher Meinung.

Wir sind hierbei jedoch anderer Ansicht als BECKER (1938). Er behauptet, daß der Phragmoplast ein typisch zytoplasmatisches Derivat sei und mit anderen Kernspindeln nicht identifiziert werden kann. Die Zytoplasmanatur des Phragmoplasten scheint mir nicht dessen wesentliche, sondern lediglich seine sekundäre Eigenschaft zu sein, da sich die überschüssige Phragmoplast-Substanz, welche bei der Bildung der Scheidewandanlage nicht verbraucht war, zu Zytoplasma verwandeln kann. Daher wird auch verständlich, daß sich der Phragmoplast nach dem Zustandekommen der Wandanlage mit Plasmafarbstoffen färbt. Aus diesem Grunde scheinen mir die Vitalfärbungsversuche BECKERS (1933, 1934) noch nicht als eindeutiger Beweis dafür zu gelten, daß der Phragmoplast aus dem Zytoplasma abgeleitet wird und daß sich die Zytokinesevorgänge ausschließlich im Plasma abspielen.

Betreffs des Zustandekommens der festen Querwand bei somatischen Zellen überzeugte ich mich, daß die Wandanlage oder Zellplatte erst durch die Berührung mit dem Zytoplasma des Mutterzellwandbelages befestigt wird. Die Phragmoplast-Substanz wird in der normalen Zytokinese bei der Entstehung der Wandanlage bloß zu einem Teil verbraucht und der übrige Teil verwandelt sich nach dem Selbstunterhalt der Tochterkerne durch Entmischung zum Hyaloplasma. Nur bei der Bildung abnormer, sehr komplizierter Querwände, deren gesamter Flächenraum auffallend die normale Querwand überschreitet wird, fast die ganze Quantität der Phragmo-

plast-Substanz zur Bildung der Wandanlage verbraucht (Fig. 35–37, 38a–40a).

Die Entmischung der Phragmoplast-Substanz tritt gewöhnlich um die Tochterkerne herum, wo die Entstehung kleiner Vakuolen vor sich geht, auf. Die entmischte Phragmoplast-Substanz verwandelt sich zum Hyaloplasma, welches sich zuerst als Vakuolenwandung, dann durch Brechen als kurze Plasmastränge oder Plasmafortsätze zeigt und später mit dem Zytoplasma zusammenströmt. Auch YASUI bemerkte, daß sich das Übrige der Phragmoplast-Substanz nicht unmittelbar nach der Vollendung der Wandanlage zur Plasmamembran an der Scheidewand der Tochterzellen umgestaltet.

Ein anderes wichtiges Ergebnis dieser Experimente ist die Bestätigung der Einzelwesenheit der Spindelfigur oder der des Atraktosoms. Die Einzelwesenheit des Atraktosoms habe ich schon in meinen früheren Arbeiten wiederholt bewiesen. Doch fällt es hierbei auf, daß sich unter der Einwirkung des Dampfgemisches die Strahlenbrechung an der Grenzfläche zwischen dem Atraktosom und dem Zytoplasma durch Quellung des ersteren ausprägt, daß seine Einzelwesenheit deutlich veranschaulicht wird, und daß es sich im angequollenen Zustande auch mit dem letzteren als unvermischbar erweist.

Auf Grund dieser Schlußfolgerung sind wir imstande zu behaupten, daß die sogenannte Kernmembran in der Prophase, also die Nicht-Mischbarkeit der Kernsubstanzen mit dem Zytoplasma in der Prophase, nie in der Metaphase verschwindet, sondern kontinuierlich als Grenzfläche zwischen dem Atraktosom und dem Zytoplasma und weiter als solche zwischen dem Phragmoplasten und dem Zytoplasma bestehen bleibt. Die Grenzfläche des Phragmoplasten verschwindet nach der Zellplattbildung erst durch die von der Umgebung der Tochterkerne ausgehende Entmischung der Phragmoplast-Substanz.

### Zusammenfassung

1. Durch Lebendbeobachtung der Staubfadenhaarzellen von *Tradescantia reflexa* wurde die Einwirkung des Dampfgemisches Ammonia-Chloroform auf die Teilungsfigur untersucht. Gleichzeitig wurden das Zustandekommen verschiedener Teilungsanomalien, die gegenseitige Beziehung zwischen den Spindelsubstanzen und der phragmoplast-bildenden Substanz, ihre Einzelwesenheit, sowohl normale als auch abnormale Zytokinesevorgänge erörtert.

2. Die Einwirkung des Dampfgemisches auf die Teilungsfigur ruft zuerst die Quellung der Chromosomen und der Spindelsubstanzen hervor, dann tritt eine Störung der weiteren Karyokinesevorgänge

ein. In der angequollenen Spindelfigur verwandeln sich die Chromosomen zur Struktur des Ruhekernes und die Spindelsubstanzen zur phragmoplast-bildenden Substanz, welche sich neben dem Chromosomenklumpen anhäuft und dort klar hyaline Räume formiert. In der Mitte des hyalinen Raumes tritt eine Wandanlage auf, welche sich unter der Mitwirkung der Zytoplasmaanhäufung um sie herum und der des Zytoplasmas vom Mutterzellwandbelag aus zur festen Querwand entwickelt.

3. Unter der Einwirkung des Dampfgemisches kommen daher Syndiploid-, Riesen-, oder Zwergkerne, zweikernige oder kernlose Zellen und vielkernige Zellen mit vielen Räumchen zustande. In der durch das Dampfgemisch hervorgerufenen zweikernigen Zelle verschmelzen die beiden Tochterkerne selten zu einem Syndiploidkern.

Zum Schluß erlaube ich mir, Herrn Ehrenprof. Dr. K. FUJII für die ständige Anregung der laufenden Untersuchungen meinen herzlichsten Dank auszusprechen. Desgleichen bin ich der Japanischen Gesellschaft zur Förderung der Zytologie für die finanzielle Unterstützung dieser Arbeit zu großem Dank verpflichtet.

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## Karyological Studies on *Iris japonica* Thunb. and Its Allies<sup>1) 2)</sup>

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(With 8 text figures)

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### Introduction

There are several karyological investigations on *Iris japonica*. KAZAO (1928) considered the sporophyte of this species as an auto-triploid having 54 chromosomes. But SIMONET (1932, 1934) reported *I. japonica* as a diploid plant with 34 chromosomes in its root-tip cells, and considered *I. japonica* var *aphrodite* as a hyper-triploid and interpreted the chromosome constitution as  $2n = 51 + 3$ , the latter 3 chromosomes being considered as derived from 3 of 51 chromosomes in *I. japonica* by fragmentation which occurred at their median constrictions.

The karyological investigation in *I. japonica* and the other 2 allied species, which were kindly put at my disposal by Dr. S. IMAMURA of Kyoto Imp. University, gave me some results different from those of the previous authors as is described below.

### Material and Method

In the fixing of the root-tip cells both NAVASHIN's and LEWITSKY's solutions were used. When fixed in the latter, the chromosomes were found longer and slender than when fixed in the former solution, but there was no essential difference in the distribution of chromosomes in the equatorial plate. NEWTON's gentian violet staining was generally adopted. Materials for the study of meiosis were fixed mostly with NAVASHIN's fluid and stained with HEIDENHAIN's iron-alum haematoxylin. Acetocarmine smears were also tried in the study of the pollen mother cells.

Drawings were made with a camera lucida.

1) Contributions from the Divisions of Genetics and of Plant-Morphology, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 231.

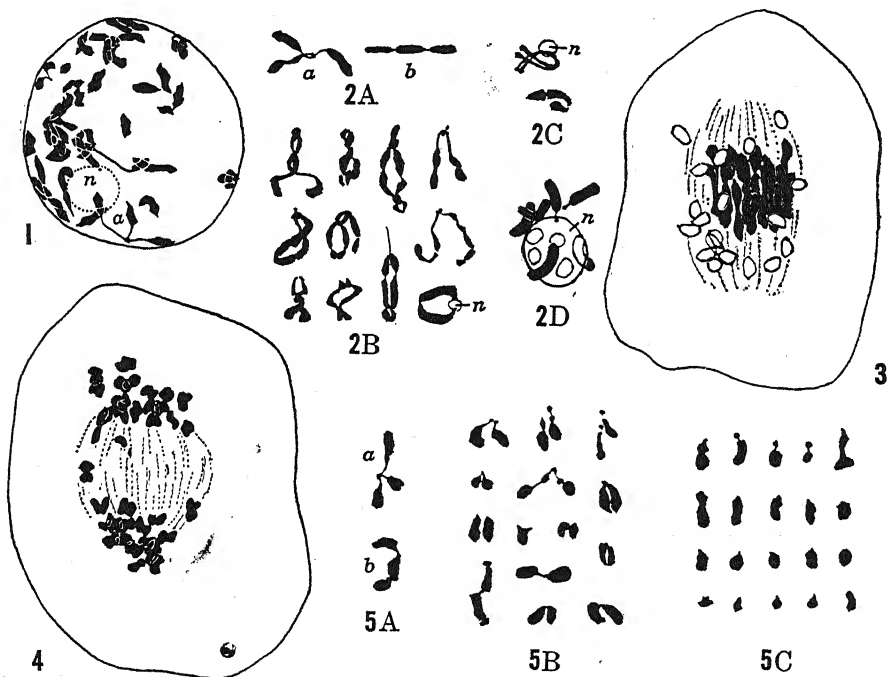
2) This work was made possible by grants from The Japan Society for the Advancement of Cytology.

## Observations

### A. *Iris japonica* Thunb.

I. *Meiosis in PMC*. There were observed 2-5 trivalents, mostly 14 bivalents, and mostly 17, sometimes nearly 17 univalents in a PMC with 54 chromosomes (Figs. 1 and 2). Three constituent chromosomes of one of the trivalents had a long slender constriction region between the short and long arms always closely associated with their short arms (Figs. 1, *a*, and 2A, *a*) in the diaphase, while the rest of the trivalents showed the end to end association (Fig. 2A, *b*).

Several chromosomes showed intimate connections with nucleoli (Fig. 2D). These connections remained until the first meiotic metaphase, and very often the relic nucleolus was seen in the spindle and also in the cytoplasm.



Figs. 1-5. *Iris japonica*. 1, diaphase nucleus of PMC. *a*, trivalent chromosome; *n*, nucleolus. 2, some chromosomes in diaphase of a PMC; *n*, nucleolus; 2A, 2 trivalents; 2B, different configurations of 11 bivalents; 2C, 2 univalents; 2D, a vacuolated nucleolus (*n*) and 6 attached chromosomes. 3, a PMC in which trivalents and bivalents (in black) are going to separate at the equator of the spindle, and the univalents (in outline) are scattering around them; some univalents behind the tri- and bi-valents are not shown. 4, anaphase of 1st meiotic division in PMC, *Drosera* type. 5, chromosomes in a diaphase nucleus of a MMC; 5A, 2 trivalents, *a*, of the same shape with that of 2A, *a*; 5B, 14 bivalents; 5C, 20 univalents. ca.  $\times 1300$ .

In the metaphase these trivalents, as well as the bivalents, formed the equatorial plate earlier than the univalents which were found scattered around them (Fig. 3). In the anaphase these trivalents generally separated into two sets, one with one chromosome and another with 2 chromosomes, and each set proceeded to the pole, together with those of the separated halves of bivalents. Generally the univalents, which came later into the equator, migrated at random into both poles without separation of their chromatids (Fig. 4). Retarded chromosomes forming miniature nuclei or extra-nuclear chromosomes were observed. Cytokinesis did not occur after the 1st meiotic division. Second meiotic division in the PMC is rather regular, though some few retarded chromosomes were found also. The miniature nuclei of the 1st division generally divided into two. After the 2nd division a simultaneous cytokinesis occurred.

Table 1. Karyotype of *Iris japonica* Thunb.

Length	Position of 1st constriction	Shape of short arm of the subterm.-constriction chromosome	Presence (+) or absence (—) of secondary constriction	Number of chromosomes			
				3 in one set	A pair in one set	One without partner	Total
L	Submedian		+	0	1	0	2
L	Subterminal	Oblong	+	0	2	0	4
l	"	Round	—	1	0	0	3
l	"	Oblong	—	0	0	2	2
l	Submedian		+	0	0	2	2
l	"		—	0	1	0	2
l	Terminal		—	0	1	1	3
M	Subterminal	Small	—	0	1	2	4
M	Submedian		+	1	0	0	3
M	Terminal		—	0	2	4	8
M	Subterminal	Round	—	0	2	0	4
M	"	Oblong	—	0	0	1	1
m	Submedian		—	0	2	0	4
S	Subterminal	Round	—	0	0	1	1
s	Terminal		—	1	2	4	11
Total				3	14	17	54

Denotations: L, very long; l, long; M, median; m, short median; S, short; s, very short.

2. *Meiosis in the megaspore mother cell (MMC)*. The behaviour of the chromosomes in the meiosis of the MMC resembled generally that of the PMC; few trivalents and the compensating number of the bivalents and the univalents (Fig. 5), miniature nuclei, extra-nuclear chromosomes in the 1st and 2nd divisions were observed. But in one case in the 1st division almost all of the univalents divided at the 1st metaphase and followed the separated trivalent and bivalent chromosomes into both poles. This may be due



to the different cellular conditions in the PMC and the MMC, which influence the behaviour of the univalent chromosomes.

3. *Chromosomes in the root-tip cells.* The chromosome number in the root tip cells of *I. japonica* is 54 as KAZAO (1928) reported (Fig. 6). We can recognize in them 3 component sets of chromosomes. Two of them resembled each other in their size and structure, but the third was of a different type, though a few chromosomes of the third group resemble those in the other 2 sets. The determination of the karyotypes in such a species as *I. japonica*, which has rather numerous and various kinds of chromosomes, is not easy. Moreover, due to the small number of the observations the karyotype shown in Table 1 may not be conclusive, but it shows the general features.

Either three nucleoli, of which 2 were larger and one smaller, or one large nucleolus were found generally in the resting or interkinetic nuclei of the root-tip cells.

### B. *Iris* sp. (Chinese origin)

This species has 36 chromosomes in its root-tip cells (Fig. 7). Two nucleoli of equal size or one large nucleolus were found in the resting or interkinetic nucleus of the root-tip cells. Karyotype structure of the chromosomes are shown in Table 2.

Table 2. The karyotype of *Iris* sp. (Chinese origin)

No.	Length	Position of 1st constriction	Shape of short arm of the subterm.-constriction chromosome	Presence (+) or absence (—) of secondary constriction	No. of chromosomes
1	L	Subterminal	Round	+	2
2	l	"	Small	—	2
3	l	Terminal		+	2
				(satellite at the distal end)	
4	M	Subterminal	Round	—	2
5	M	Submedian		—	2
6	M	Subterminal	Small	—	2
7	M	"	"	—	2
8	m	Submedian		+	2
9	m	Terminal		—	2
10	m	Subterminal	Small	—	2
11	m	"	Round	—	2
12	m	"	"	—	2
13	m	"	"	—	2
14	S	"	"	—	2
15	S	"	"	—	2
16	s	Terminal		—	2
17	s	Submedian		—	2
18	s	Terminal		—	2
Total					36

Denotations same as in Table 1.

C. *Iris formosana* Ohi

This species has 28 chromosomes in the root-tip cells. Compared with the other two species, however, it has a very different chromosome constitution (Fig. 8). Two smaller nucleoli of equal size or



Figs. 6-8. Equatorial plates in root-tip nuclei. 6, *Iris japonica*; 7, *Iris* sp. (Chinese origin); 8, *Iris formosana*. ca.  $\times 1270$ .

one larger nucleolus were found in the resting and interkinetic nuclei in the root-tip cells. The karyotype diagnosis is shown in Table 3.

Table 3. Karyotype of *Iris formosana* Ohi

No.	Length	Position of 1st constriction	Shape of short arm of the subterm.-constriction chromosome	Presence (+) or absence (—) of secondary constriction	No. of chromosomes
1	L	Submedian		+ (at the middle part of long arm)	2
2	L	„		+ (at the middle part of long arm and a satellite at the distal end of short arm)	2
3	L	„		+ (at the distal part of the long arm)	2
4	L	Subterminal	Small	+ (satellite at the distal end of short arm)	2
5	L	„	„	—	2
6	l	„	Round	+ (in the long arm)	2
7	l	„	„	—	2
8	l	„	„	—	2
9	l	Subterminal	Small	—	2
10	M	Submedian		—	2
11	M	„		—	2
12	M	Subterminal	Round	—	2
13	m	„	Small	—	2
14	m	Submedian		—	2
Total					28

Denotations same as in Table 1.

## Discussion

**1. Derivation of *I. japonica* Thunb.** "Syaga" (Japanese name) which was described by Thunberg (1794) as *I. japonica* is a wild indigenous plant in this country, being distributed from Kyūsyū to northern Hontō and Sikoku, but not found in Hokkaidō, Ryūkyū, Taiwan, and Tyōsen. An allied plant, "Kotyōkwa", was already known in China, which does not grow wild in Japan.

*I. japonica* growing in Japan has 54 somatic chromosomes in its root-tip cells. My observation in the meiosis of the PMC as well as of the MMC show that 1) there are very few trivalents, 2) about one third of the chromosomes remain as univalents, and 3) the remaining, mostly 14 pairs of the chromosomes, are associated as bivalents. From these data and the chromosome behaviour in the meiosis, it seems that the species in question is not an autotriploid as was considered by KAZAO at that time, but an allotriploid, which was derived from a hybrid between 2 species having different karyotypes, and in which the chromosome set of one parent has been doubled. The chromosome studies in the root-tip cells gave sufficient evidence; namely the chromosomes can be divided into 3 sets, 2 of them much resembling each other, while the third has only a few chromosomes resembling those in the other 2 sets.

Consequently SIMONET's *I. japonica* can not be identified with *I. japonica* ("Syaga"), because the former is a diploid plant having 17 haploid chromosomes. *I. japonica* var. *aphrodite* having 54 somatic chromosomes (SIMONET, 1934) might be a variegated *I. japonica* ("Syaga") probably being mutated vegetatively but is not the direct derivative of SIMONET's *I. japonica*.

My Chinese material is a diploid plant having 36 somatic chromosomes in its root-tip cells. This number may suggest an intimate relation of this species to *I. japonica*, but its karyotype does not exactly coincide with either of the karyotypes of *I. japonica*. Another allied species, *I. formosana*, which is known to grow only in a limited area in the southern part of Formosa, is also a diploid plant having only 28 chromosomes. Not only the karyotype but also the chromosome number is different from those of other species.

Thus we cannot find out the parent plant of *I. japonica* among those diploid species. *I. gracilipes* is a diploid plant (KAZAO, 1928) which has 36 somatic chromosomes, but its morphological characters are so different from those of *I. japonica* and other allied diploid species as to permit the suggestion that it has direct relationship with *I. japonica*. From these data and the isolated habitat of *I.*

*japonica* it may be concluded that *I. japonica* originated by hybridization and propagated in the present habitat with its hybrid vigour, while the parent species have become extinct.

Indeed this plant, *I. japonica*, produces more than 5 stolons beneath each rosette and the full grown stolon attains a length of as much as half a meter and form a rosette on the tip.

**2. The aneuploidy.** Genus *Iris* is one of the remarkable groups of plants in which the aneuploid series are found, though in some sections there were found only one kind of chromosome number or a simple polyploid series (SIMONET, 1932, 1934). *I. japonica* and its allies show an aneuploid series and also a triploid too, namely they have haploid chromosome numbers as 14, 17, 18, and  $54/2$ .

On the cause of production of such an aneuploid series several views have been already proposed, i.e. due to the addition of chromosome sets, fragmentation, fusion, translocation etc. The absence of the terminal-constriction chromosome in the karyotype of *I. formosana* which has the smallest chromosome number and longer chromosomes among the allied species, and its presence in the latter may show that the numerical increase of the chromosomes in the latter species is due to fragmentation which occurred on the attachment point. The 1st constriction of chromosomes in these species is often very wide, so that the 2 arms are very remote from each other; this sometimes leads to miscounting. So that SIMONET's suggestion of fragmentation is plausible, though I can not agree with him in his suggestion as to the derivation of *I. japonica*. He suggests the fragmentation of the median-constriction chromosome, but the length of those chromosomes which have terminal attachment points varies, and very small terminal-constriction chromosomes in *Iris* sp. (Chinese origin) may suggest the fragmentation of a subterminal-constriction chromosome, though they can be confirmed only after the examination of the homology among those supposed fragmented chromosomes.

At any rate an important fact should be recognized, i.e. those natural plant groups having aneuploid chromosome series are mostly perennial plants and they can more or less propagate vegetatively. Therefore when such an aneuploid plant once appeared, it may remain viable among those parent plants or can survive even after the latter became extinct. On the contrary, the aneuploid plants which propagate only by seeds may be eliminated easily through meiosis. I can show an example in my experiments with *Papaver*. In the  $F_1$  plant of *Papaver somniferum*  $\times$  *P. lateritium* the annual character is dominant over the perennial character, and the derivative of the back crosses with *P. somniferum* were all annual. However, in the  $F_1$  plant of *P. somniferum*  $\times$  *P. orientale* the perennial is dominant

over the annual and the derivatives of the back crosses with *P. orientale* are all perennial. In both cases I have had several aneuploid due to the differences of the chromosome numbers among parent plants, and in the case of the *P. somniferum*  $\times$  *P. lateritum* those aberrant chromosomes were eliminated through meiosis, and after 2 or 3 generations all derivatives returned to *P. somniferum* (YASUI, 1926); but in the case of the *P. somniferum*  $\times$  *P. orientale* those aneuploid plants are growing well through many years and propagating vegetatively (YASUI, in press).

In the field, the aneuploidy may appear occasionally in any plant by chromosomal aberration and very often in those plant groups in which a more or less polyploid chromosome series is present due to the hybrid formation, and they live and make, an aneuploid series when they have power to propagate vegetatively; otherwise they will be eliminated through the meiosis.

### Summary

1. *Iris japonica* Thunb. (Japanese name "Syaga") is an allotriploid ( $2n = 54$ ), but neither autotriploid nor hypertriploid. It contains 2 different karyotypes; the chromosome constitution may be described as AAB.

2. *I. japonica* has been probably derived from a hybrid between 2 species which had different karyotypes, probably A and B, and one set (A) of the parent chromosomes was doubled. It might have originated partly in its present habitat and survived there, while the parent species have become extinct.

3. A Chinese species allied to *I. japonica* is a diploid plant having 36 somatic chromosomes. Its karyotype (C) is different from A and B. Neither this species nor SIMONET's *I. japonica* is to be considered as the direct parent of our *I. japonica* Thunb.

4. *I. formosana* Ohi is also a diploid plant having 28 somatic chromosomes. Not only its karyotype (D) but also the number of the chromosomes are different from those of other species. This type may be intimately related to the ancestral type of other species. The absence of terminal-constriction chromosomes in this karyotype and their presence in other species suggests that the increase of the chromosome number in the latter is mostly due to the fragmentation of the chromosomes which occurred at the attachment constriction.

5. *I. japonica* and its allied species show an aneuploid series,  $2n = 28, 34, 36$ , and an allotriploid  $2n = 54$ . They all show different karyotypes.

6. Aneuploidy may appear occasionally in any plant, but in that which propagates by seeds only, the aberrant chromosomes will be easily eliminated through meiosis. Therefore the survival of the aneuploid series in nature is probably due to the capability of vegetative propagation in these plants.

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## Studies in the Indian Scilleae. II. The cytology of *Scilla indica* Baker.

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(With 36 figures in the text)

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### Contents

	Pages
Introduction . . . . .	189
Materials and methods . . . . .	190
Somatic chromosomes. . . . .	191
Meiosis . . . . .	193
Discussion . . . . .	198
a. Polyploid gametes. . . . .	198
b. Somatic pairing. . . . .	200
c. Chromosome bridges . . . . .	200
d. General considerations . . . . .	201
Summary . . . . .	203
References . . . . .	204

### Introduction

The *Scilleae* has been a favourable tribe for karyological investigation, not only on account of the comparatively large size of the chromosomes but also because of the different karyotypes met with in genera like *Scilla*, *Ornithogalum*, *Hyacinthus*, *Urginea* and *Bellevalia* comprised in the tribe. Various basic chromosome numbers have been reported for these genera by many authors. For example in a previous paper Raghavan (1935) reported the diploid number of *Urginea indica* to be twenty. The occurrence of consecutive haploid chromosome numbers has been reported in several genera of the Scilleae. Delauney (1927) has reported 6, 7, 8, 9, 12 and 17 as the haploid numbers in *Ornithogalum*. 4, 8 and 9 have been reported for *Bellevalia* (Levitsky and Tron 1930), and for the genus *Scilla* itself Satô (1935) has reported haploid numbers of 6, 8, 9, 10 and 17. Karyotype analysis of various species and varieties has very often helped in the clarification of the method of origin of these consecutive chromosome numbers. Most commonly these are the result of fragmentation and elimination of paired chromosomes and hybridization.

An analysis of the karyotypes of a number of species of *Scilla* being available, it was thought that a karyotype study of this species would throw some light on the origin of this chromosome number. The chromosome number of *Scilla indica* has not so far been recorded, and according to Hooker's Flora of British India only two species of *Scilla* occur in a wild state in India, *Scilla indica* and *S. Hoenakeri*. We have not been able to get bulbs of the latter. The somatic chromosome complement of the closely allied and most commonly occurring *Urginea indica* having already been analysed (Raghavan 1935), it would be of interest to compare the two karyotypically and see if any clue could be gained regarding the relationship between the two, and the probable method of origin of *Scilla indica*.

During the course of the investigation three distinct karyotypes of *Scilla indica* were met with,  $2n = 44, 45$  and  $46$  and this added to the interest. Meiotic stages were also studied in some detail and because of the extreme irregularity, there is no benefit in describing the meiosis in the three types separately.

### Materials and methods

Bulbs were got from the following sources and grown separately in the University Botanical Gardens. Extreme care was taken to avoid mixing those from one locality with those of another.

1. Bulbs were collected in a wild state from Saidapet Teachers' College compound, and Adyar, Madras.
2. Some were got from the Soundarya nursery, Madras.
3. Some bulbs were received from the Agri-horticultural gardens, Madras.

The roots were grown in sand or in water culture and fixed in a modification of Navashin's fluid evolved in this laboratory, in which the proportion of formalin was reduced by about five per cent. The short schedule was found to be more satisfactory, by which fixed materials were rinsed in water and then washed in 50% alcohol for a few hours and hardened in 70% overnight. Prefixation in Carnoy's fluid was found desirable in aiding proper fixation and the paraffin sections were cut at a thickness ranging between 12–20 microns.

The required stages of pollen development were determined by acetocarmine smears and the anthers were fixed also in Navashin after prefixation in Carnoy. Smears were also made and fixed in Belling's Navashin. In all cases of prefixation with Carnoy, staining had to be prolonged to an hour a half. The materials got from



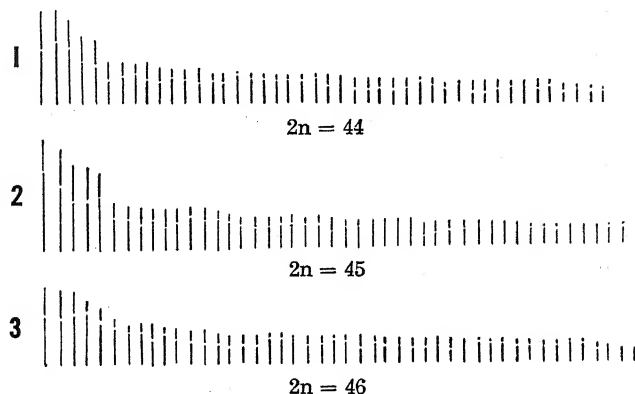
the Agri-horticultural gardens of Madras were of two kinds. In one, the leaves were broader ('broad-leaved') than in the other ('narrow-leaved'). The average length of the leaves in both the types was about eight inches. The breadth, however was variable; in the 'broad-leaved' variety it was  $1\frac{1}{4}$  to  $1\frac{1}{2}$  inch and in the 'narrow-leaved' it was about  $\frac{1}{2}$  an inch. In both, however, the dark blue patches characteristic of the species are present. The materials got from the Soundarya nursery were identical with the 'broad-leaved' variety described above, while those collected at Adyar and Saidapet conformed to the 'narrow-leaved' type.

### Somatic chromosomes

This important morphological difference was found on cytological examination, to be associated with a difference in the chromosome number. Root-tip examination of a dozen bulbs of the 'broad-leaved' variety revealed that the diploid chromosome number was 45. Among the 'narrow-leaved' variety on the other hand, there were two karyotypes, 44 and 46. There was however no morphological difference between these two karyotypes. Figs. 4-7 are the somatic plates of the three karyotypes.

Figs. 1-3 represent the idiograms of the three karyotypes. Analysis of the complements is rendered difficult firstly by the largeness of the number of chromosomes, and secondly by the lack of distinct morphological differences between the members of the complement and the consequent intergrading.

In both the 44 and the 45 varieties there are five long chromosomes. The



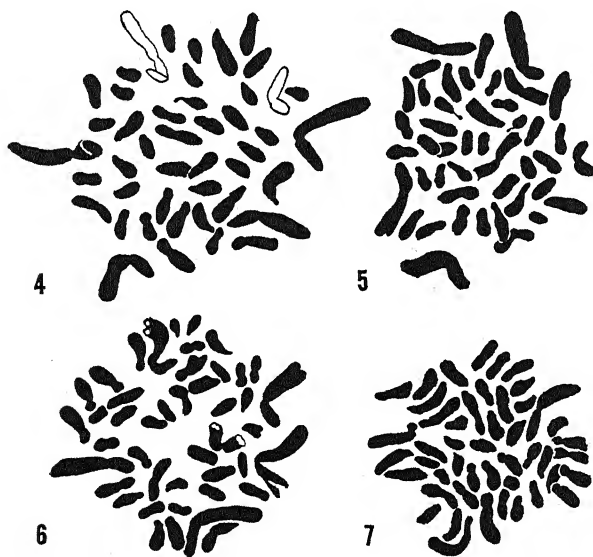
Figs. 1-3. Diagrammatic representation of the somatic complements of the 44, 45 and 46 types.

longest chromosome is about 6.5 microns in length, and the size of the five long chromosomes range between 6.5 to 4.3 microns. The medium chromosomes are from 3.5 to 2 microns while the short chromosomes are 1.8 and less.

In the 44-type, out of an average total chromosome length of about 105 microns the length of the five long chromosomes constitutes about 28 microns. In the 45-type, the corresponding figures are 106 and 26. In the 46-type also the same proportion is kept up approximately.

All the five long chromosomes are not of the same size; three of them are of approximately equal length while the other two are slightly shorter. The drop between the long and the medium chromosomes is more or less sudden, while the rest of the chromosomes can be arranged in a very gentle descending order in respect of their size. No sharp line can be drawn in the matter of their length. Regarding the constrictions, the five long chromosomes have a submedian attachment constriction, and the medium chromosomes have also the same type of constriction generally. But among the short chromosomes both sub-terminal and terminal attachment constrictions occur.

Of the short chromosomes with terminal constriction, two have satellite at their proximal end. Satellite chromosomes could however be seen only in a few plates. In fig. 5 which is a 45-complement, there is a pair of Sat.-chromosomes, while in fig. 4 only one is seen. In a number of plates a pair of chromosomes would not take up the stain. It is of interest to record that this



Figs. 4-7.  $\times$  ca. 2500. Somatic metaphases from the root tips of *Scilla indica* (broad leaved and narrow leaved varieties). 4. Broad leaved  $2n = 45$ . Note two chromosomes one long and one medium that would not take up stain. 5. Do. Note the two satellited chromosomes. 6 and 7. Narrow leaved,  $2n$  44 and 46 respectively.

phenomenon was confined to the 45-karyotype and though a number of the 44 and 46 types were examined, none of them showed it. Of these two chromosomes, one belongs to the long variety and the other to the medium (fig. 4). Figs. 6 & 7 represent somatic plates of 44 and 46-karyotypes.

## Meiosis

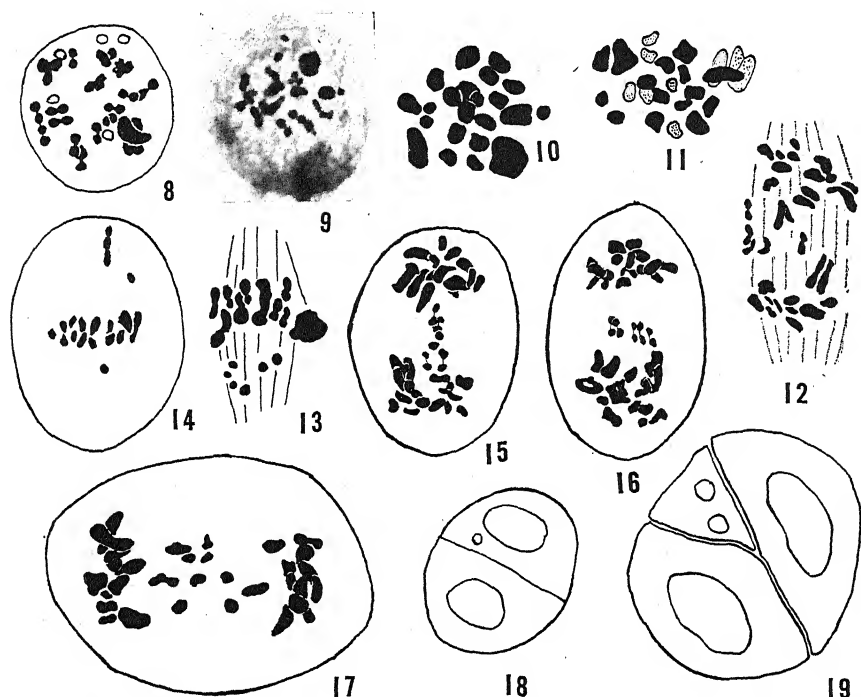
Stages earlier than diakinesis were not studied because of the delicateness of the chromosome threads. The chromosomes at diakinesis show varying degrees of multivalent formation. In the majority of the cells they were found as quadrivalents, trivalents, bivalents and univalents in varying proportions. Fig. 8 represents a pollen mother cell in late diakinesis with one quadrivalent, five trivalents, ten bivalents and five univalents. The nucleolus is not shown. Quadrivalent formation was however of comparatively rare occurrence. Out of over fifty cells examined, only in two were quadrivalent association prevalent. Two showed, six trivalents, ten bivalents and six univalents. The commonest mode of occurrence was eight trivalents, eight bivalents and four univalents. Fig. 9 shows a still later stage, where the multivalent-formation is clearly seen.

The configuration assumed by the trivalents was more or less uniform giving a morphologically recognizable form. Three of the long chromosomes form generally a trivalent whose configuration on account of their size is somewhat distinct from the trivalents formed either by the short or the medium chromosomes. The two cases of the quadrivalents were of the linear rod type; also quadrivalent formation is restricted to the medium or short chromosomes.

At metaphase I, the chromosomes are very much condensed. The number of bodies to be seen at first metaphase is naturally variable. In fig. 10, 22 bodies are seen while in fig. 11, 24 can be recognised. There were others showing 24, 20 etc. In a few as many as 25 bodies are recognised. Obviously in those exhibiting a greater number of bodies, there should have been a greater number of univalents and bivalents with a proportionate reduction in the number of trivalents. In side views (fig. 13) the trivalents and bivalents arrange themselves on the equator in a haphazard manner, while the univalents lie outside the spindle nearer the poles. In fig. 13 six univalents are seen all towards one pole while in fig. 14 one on either side of the spindle is seen. In the same fig. 14 can also be seen a trivalent towards one pole. Such a relegation to the pole, of a trivalent was, however of rare occurrence. It must be regarded as a case of a trivalent reaching a pole without disjunction.

The spindle during anaphase presents a variety of appearance. Most commonly the whole spindle is covered with chromosomes stretching from one pole to another (fig. 12). The trivalents usually disjoin during anaphase, one of the components travelling to one pole, while the other two reach the other pole. The behaviour of

the univalents is rather irregular. While their configuration in fig. 15 suggest their reaching the respective poles in an undivided condition their form in fig. 16 conveys the impression that there is a likelihood of their division. In fig. 16 all the five univalents are in the equator, while in fig. 15 it looks as if three of the univalents are reaching one pole, while the other two are reaching the opposite pole. The only other interpretation possible is that these may be taken as ordinary rod bivalents formed by some of the short chromosomes. Why these bivalents, formed presumably in a regular manner, should lag in the equator will be hard to explain, and their reaching the pole without division makes it more probable that these are univalents rather than bivalents.



Figs. 8-18.  $\times$  ca. 1950 except fig. 17 which is ca. 2625. 8. Pollen mother cell in late diakinesis showing one tetravalent, five trivalents, ten bivalents and five univalents. 9. Photomicrograph of pollen mother cell in late diakinesis showing univalents, bivalents, trivalents and quadrivalents.  $\times$  ca. 1320. 10 and 11. MI showing 22 and 24 bodies respectively. 12. First anaphase, the whole spindle being covered with chromosomes stretching from one pole to another. 13. Side view MI. Univalents lying outside the spindle nearer the pole. 14. Two univalents and one trivalent lying outside the spindle. 15 and 16. First telophase with five univalents lagging. In 15 two are reaching the top pole while three reach the bottom. In 16 all the five are in the equator. 17. Do. with a number of laggards. 18. Wall formation after the first division and also the organisation of a micro-nucleus. 19. Three-celled condition after the first division. Two micro-nuclei have organised themselves into a third cell.

While it is usual for the univalents to be included in the daughter nuclei without division, cases have been recorded of occasional division of univalents, and the daughter halves going to the opposite poles. Ramanujam (1937) has reported a similar behaviour of univalents in his triploid rice. Frequently lagging bivalents divide late in the spindle and get lost in the cytoplasm. Occasionally, however, these straying chromosomes form a membrane round them and constitute micronuclei (fig. 18). In one case there were three cells constituted after the first division (fig. 19). Presumably these chromosomes outside the spindle have not only organized themselves into a distinct nucleus but a cross wall has been formed cutting it off as a separate cell altogether. Levan (1936) has also recorded the formation of three micronuclei in the triploid *Allium Schoenoprasum*. "Dies ist der Fall in Fig. 37c, in der 3 Kleinkerne eine besondere Zelle bilden". Here two such nuclei have built up a third cell. He has also recorded that when these undergo the second division six cells result. Such a division was not observed in the present material. Presumably these degenerate. Since there is extensive pollen degeneration (pollen viability being about 25%), it is likely that such cells undergo degeneration. Fig. 16 shows telophase with a number of laggards.

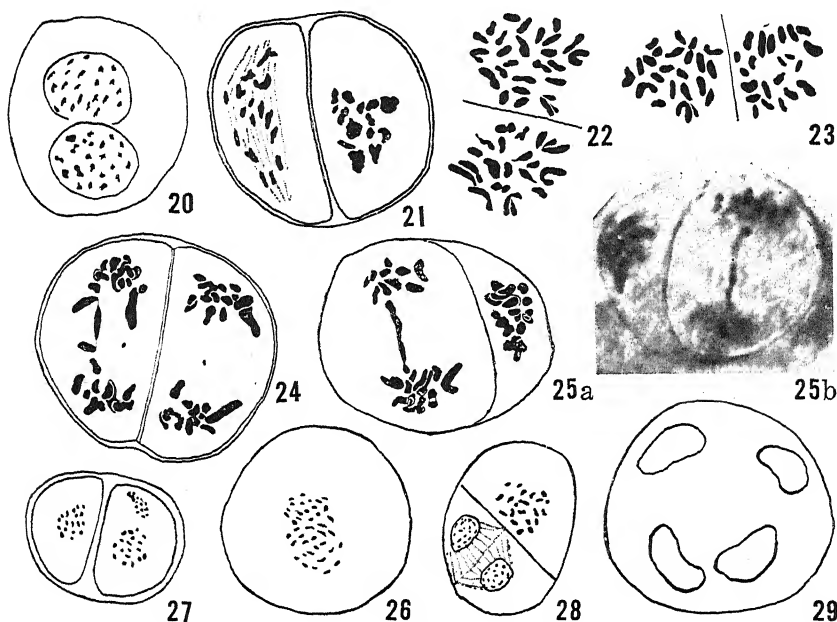
Normally a cross wall is formed at the end of the first division. But occasionally as in fig. 20 we find two interphase nuclei with no cell wall between them. The chromosomes also show a marked split obviously for the second division. In fig. 26 there are two M II plates lying together there having been formed no cross wall at all. It may be expected to function as a diploid cell.

Division II is somewhat more regular. In some, however, there are lagging forms constituting what may be termed chromosome bridges (fig. 25a and b). In some again, the chromosomes instead of arranging themselves in the equator, are distributed throughout the spindle (fig. 21). This phenomenon, as already stated, was found in division I also. While in the latter case as Darlington (1938) observes, it may be due to the asymmetrical nature of the trivalents, in which the presence of the three centromeres interferes with the normal orientation of compound bodies on the equator, in the former case it may be due to the disparity (timing unbalance) between the activity of the spindle and the centromere. The second division is as a rule simultaneous in the two daughter cells. In a few cases, however, some difference is seen in the relative activity of the two daughter cells. In fig. 28 while one daughter cell is in the second metaphase, in the other the second division is almost complete, even wall formation having been accomplished. In a few

exceptional cases there is no evidence of wall formation at all. For instance fig. 29 shows pollen mother cell in the second telophase, but there is no indication of any wall at all.

In some cases (fig. 27) three groups of chromosomes were noticed at metaphase II.

Figs. 22 & 23 show a number of second metaphase plates exhibiting varying numbers of chromosomes.



Figs. 20-29.  $\times$  ca. 1950, except figs. 27 and 28 which are  $\times$  1260. 20. Two interphase nuclei without any cell wall between them. Note also that the chromosomes show a marked split presumably for the second division. 21. The distribution of chromosomes throughout the spindle in the second division. 22 and 23. Second metaphase polar view showing different numbers at the poles. 24 and 25a. Second telophase. Note the chromosome bridges and the fragments in 24. 25b. Photomicrograph of the same as 25a. 26. Two MII plates lying together, with no cross wall between them. 27. Three groups of chromosomes are seen at MII. 28. Non-simultaneous second division. One cell is in second metaphase while in the other the second division is almost complete including wall formation. 29. Second telophase with complete suppression of cross wall formation.

Owing to the occurrence of multivalents and univalents in meiosis, all the pollen grains did not possess the  $n$  number of chromosomes. There occurred proximate numbers also. Counts were made in thirty six grains and the results are given in an analysed form in the accompanying table.

Figs. 30 & 31 show pollen grains with 22 and 25 chromosomes respectively. In fig. 32 is shown a normal haploid pollen with 23

chromosomes, while in fig. 33 is shown a diploid grain with 46 chromosomes.

Table 1

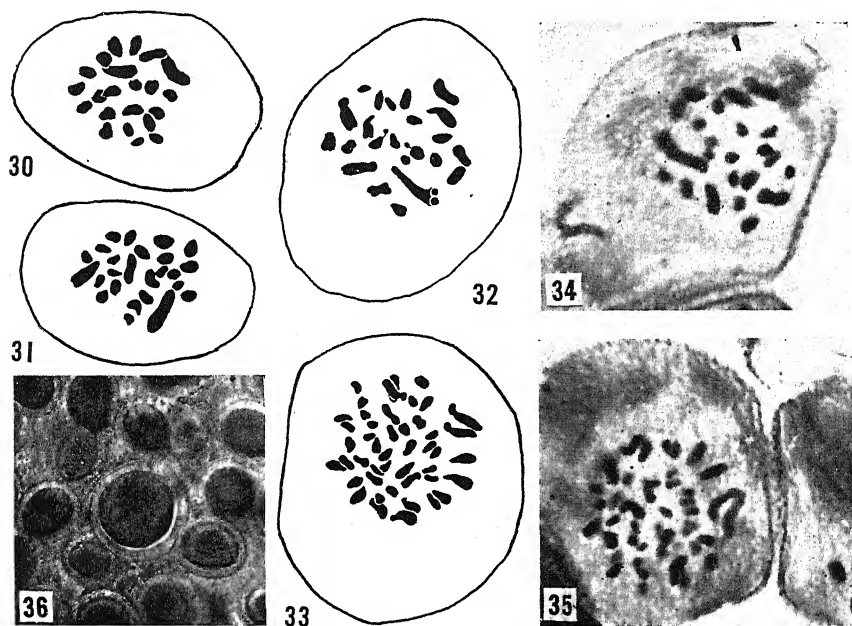
No. of chromosomes	17	18	19	20	21	22	23	24	25	26	27	28	29
No. of cases	1	2	—	2	3	4	9	3	4	5	1	—	2

Standard deviation ( $\sigma$ ) = 2.7.

Arithmetic mean = 23.3.

$\sigma = \sqrt{\frac{\sum D^2 f}{n}}$  where  $D$  is the deviation of each class value from the mean of all varieties;  $f$  is the frequency of each class,  $n$  = total no. of variates (individuals).

The numbers range from 17 to 29 and the mode is 23—the haploid number. Fig. 34 is a photomicrograph of a pollen grain with 23 chromosomes. Occasionally a few pollen grains were found to contain the full diploid set of chromosomes. Fig. 35 shows photo-



Figs. 30-31. Pollen grain in post-meiotic metaphase.  $\times$  ca. 1950. 30, showing 22 chromosomes, 31 showing 25 chromosomes. 32. Pollen grain containing the full haploid number of chromosomes 23.  $\times$  ca. 1950. 33. Diploid pollen grain showing 46 chromosomes.  $\times$  ca. 1950. 34. Photomicrograph, a haploid pollen grain ( $n = 23$ ), same as fig. 32.  $\times$  ca. 1900. 35. Photomicrograph of the same material as the fig. 33. Note the arrangement of the chromosomes in pairs. By its side the haploid pollen grain shown in fig. 34 may be seen in part. Note that there is practically no difference in size between the haploid and the diploid grains.  $\times$  ca. 1900. 36. Photomicrograph, a giant pollen grain surrounded by ordinary pollen grains.  $\times$  ca. 520.

micrograph of a grain with 46 chromosomes from that particular karyotype. These were slightly bigger than the normally formed ones. It can be seen very clearly that the chromosomes exhibit definite 'somatic pairing'.

There is extensive degeneration of pollen and there is dimorphism of pollen grains. In fig. 36 is seen a photomicrograph of a giant pollen grain surrounded by smaller grains. Unfortunately it was not possible to get the post-meiotic chromosome number in the giant pollen grains, as these occurred only few and far between, and as the plants had ceased to flower. The diploid pollen grains described earlier do not differ very much in size from the ordinary ones to merit their distinction as giant pollen. Giant pollen grains having twice the usual number of chromosomes were observed in *Allium carinatum* and *A. oleraceum* by Levan (1933). Here, however the diploid grains are not so very much bigger than the ordinary grains as to be called giant pollen grains. This suggests that these giant pollen grains whose chromosome number was unfortunately not determined, are different from the diploid grains. It is likely that these are polyploid gametes containing  $3n$  or  $4n$  of chromosomes. This point, it is hoped may be cleared, when the plants next flower.

### Discussion

#### a) Polyploid gametes

Polyploid gamete formation is fairly of common occurrence in certain genera and species. Recently the Imperial Bureau of Plant Genetics, Cambridge (1936) has published a summary of the literature relating to the experimental production of haploids and polyploids. In that connection the formation of polyploid gametes has also been treated. Ellison (1937) has described three methods by which polyploid gamete formation may occur. Firstly the pollen mother cell at Metaphase I had a giant nucleus containing polyploid chromosome numbers arising out of nuclear fusion. Secondly from a normal pollen mother cell, in which due to the absence of a dividing cell wall fusion of the two M II plates took place. Thirdly from a normal pollen mother cell in which there was a complete failure of cell wall formation so that the whole pollen mother cell containing four nuclei each with the haploid number of chromosomes became a giant pollen grain.

One feature that appears common to all these three methods is the failure of cell wall development. This failure may arise during the separation of the individual pollen mother cell as in the first



method, or at some subsequent stages as in methods two and three. In the third method the two meiotic divisions have proceeded normally.

In the present investigation giant pollen grains have been seen occasionally (fig. 36). It has not been possible to determine their chromosome number, though it is very likely that they are polyploid. There have also been found a few pollen grains slightly bigger than the normal haploid gametes, in which the full diploid complement is found (figs. 33 & 35). The chromosome numbers in the normal grains range from 17 to 29 and this is due to the extremely irregular meiosis consisting of the formation of multivalents and their irregular behaviour at disjunction and the ununiformity of the behaviour of univalents' migration and division. But the formation of the gametes with the full diploid complement and the occurrence of the chromosomes in pairs must have resulted from one of the causes specified above connected with wall formation.

Among the abnormalities recorded here, are the following. Firstly due to the absence of a dividing cell wall, fusion of the two M II plates took place (fig. 26). Secondly there was suppression of wall formation in the two divisions, so that four-nucleated grains were found (fig. 29). In the first type, the diploid plate should again divide homotypically and two grains with the diploid number must result. Such pollen dyads, however, were not found in the present investigation. And these pollen grains, the few that occurred, were found singly without any sign of its sister grain. It is therefore very likely that there is rather a suppression of division II, than a dyad formation. Levan (1933) has recorded giant pollen in *Allium carinatum*, and in *A. Schoenoprasum* (1936). He believes (1936) that the mechanism of the formation of these giant pollen grains consists in the omission of the second meiotic division. He terms this a "monokinetic meiosis". This may also explain to a certain extent the somatic pairing exhibited by these chromosomes.

In the second of the abnormalities recorded, there was omission of wall formation in the two divisions. Naturally the giant pollen grain resulting from a fusion of these four nuclei should possess the tetraploid number. Unfortunately no such polyploid gametes were found. Nor could the chromosome number of the pronouncedly big 'giant' pollen grain be determined. But the occurrence of this abnormality, however occasional, implies unmistakably that such tetraploid gametes must be present. To our mind, these giant grains may be those that have arisen from an abnormality such as the one indicated above.

### b) Somatic pairing

A word may be said of the paired occurrence of chromosomes in the diploid gametes. Somatic pairing is an expression of the affinity between the parental chromosomes. They do not come into actual contact as in meiosis. Simple diploids can, on this assumption, show somatic pairing. Of the favourable conditions for somatic pairing, the phenomenon of doubled nuclei is the commonest. On account of the failure of the divided chromosomes to separate, the chromosomes probably remain together at the resting stage and this consequently facilitates pairing in the succeeding metaphase. Huskins and Smith (1932) found somatic pairing in *Sorghum* to result from the above named cause. It may also be added that somatic pairing was observed in the root tip cells of *Urginea indica* (Raghavan 1935). It would appear that in the pollen grain similar reason must account for this pairing. The failure of wall formation and the suppression of the second division amount to the formation of doubled nuclei in which the chromosomes, as in ordinary somatic cells, had probably remained together and so were able to pair in the post-meiotic metaphase.

### c) Chromosome bridges

The occurrence of bridges at first and second anaphase is a phenomenon of frequent occurrence. Bridges at second anaphase are more common than in the first divisions. This is an indication of structural hybridity. An organism which may be a hybrid in respect of a number of genes or in respect of the presence or arrangement of segments of its chromosomes, is known as a structural hybrid (Darlington 1929). Genetically the crossing over in the dislocated segments produces lethal combinations. Cytologically the results of pairing and crossing-over can be observed.

According to Upcott (1937) structural changes may be divided into two groups. Eu-centric changes involving no alteration in the linear order of the genes in relation to the centromere—these do not give rise to bridges and fragments. The other, inverted or dys-centric changes which involve alteration in the linear order of the genes and give rise to bridges and fragments. The occurrence of the bridge in the first or the second anaphase, is according to her, governed by the position of the chiasmata in relation to the centromere. Proximal chiasmata lie between the centromere and the inversion. Inversion chiasmata lie within the inversion, and distal chiasmata lie beyond it. If a single chiasma is formed in the inversion, a bridge occurs at the first anaphase. If in addition to this a proximal chiasma is formed, the bridge may be at the first or the second division.

In the material under investigation, bridges occur very frequently in the second division (fig. 25a and b). In fig. 24 is seen also a fragment by the side of the bridge. This implies that a short inversion, has according to its position, given rise to long bridges and very short fragments. Presumably the dislocated segment lies nearer the distal end of the chromosome.

There cannot be any doubt whatsoever that some chromosomes have undergone structural change. Even in somatic plates we find that some of the recognizable types of chromosomes have no exact homologues. This means that structural change has occurred.

#### d) General considerations

It would appear that *Scilla indica* is a polymorphous species more or less widely distributed in India. There are only two species of *Scilla* said to be indigenous to India, of which this is the more common.

Three distinct karyotypes are met with;  $2n = 44, 45$  and  $46$ ; whereas  $44$  and  $46$  types are morphologically indistinguishable, the  $45$  type is characterised by distinctly broader leaves. The  $45$  types were got from nursery gardens and it is likely that these are garden varieties and that the  $44$  and  $46$  types represent the wild.

The analysis of the karyotypes, though rendered difficult by the largeness of the number of chromosomes and also by the lack of any distinct morphological peculiarity of the chromosome, revealed none the less, the presence of at least five longish chromosomes of which three are particularly long, and of equal length approximately. The rest of the chromosomes merge into one another in respect of their size. From the chromosome morphology it looks as though the somatic complement of *Scilla indica* is composed of a diploid set of some species with twenty chromosomes and a triploid set of a species with sixteen chromosomes.

The three prominently long chromosomes are always characterised by a sub-median attachment construction. It is likely that the diploid complement of some species of *Scilla* having two prominently long chromosomes with sub-median centric constrictions in its diploid complement, may have entered into the karyotype composition of *Scilla indica*.

The karyotype analysis of different species of *Scilla*, made available by the work of Satô (1935), gives an indication of what this diploid species is likely to be. Of the nine species that he has examined, the karyotype of *Scilla peruviana* comes nearest to the diploid constituent of the somatic complement of the species under investigation. The diploid complement has a pair of very prominent

long chromosomes with median constriction and there is a sudden drop in the size of the rest of the chromosomes. There are three pairs of very short chromosomes two of which have sub-terminal constriction while the other has terminal constriction with a satellite at their proximal end. Some other species of *Scilla*, say something like *S. hyacinthoides* ( $2n = 20$ ) may be the other constituent.

Some interesting observations would be made in respect of the size of the chromosomes in the different complements. The size of the chromosomes goes on decreasing as the number of chromosomes in a complement increases. While for example in a twelve-chromosomed species (*Scilla siberica*) the range of size of chromosomes is between 28.8 microns and 15.6, in a twenty chromosomed species like *S. hyacinthoides*, it is from 10.1 to 4.3 microns. In forms like *Scilla japonica* one of whose karyotypes shows 42–44 chromosomes, it has been suggested that the karyotype may have a diploid set of the species with 16 chromosomes and a triploid set of the species with 18 chromosomes. If the size of the chromosomes composing this karyotype is analysed, it is found that these very chromosomes of the 16-chromosomed and 18-chromosomed species have become considerably smaller in the 44-karyotype, which as has been said already, is made up of these chromosomes. It seems, therefore, reasonable to infer that the chromosomes entering into the formation of a hybrid tend to become considerably smaller in the latter, especially when the parental chromosomes happen to be in unequal sets, as in the cases cited above. This same phenomenon may explain to some extent the diminution in size of the chromosomes of *Scilla indica* compared to the chromosomes of the 16 and the 20-karyological type.

The method of formation of this karyotype ( $2n = 44$ ) must, on the hypothesis presented, be by a sort of hybridization between a 16-chromosomed and a 20-chromosomed species. Triploids occur frequently in the *Scilleae*. Triploidy has already been reported in the closely related *Urginea indica* (Raghavan 1935). Triploids are almost always self sterile. Hybridization between a triploid (unreduced gamete from a triploid) and a diploid (unreduced gamete from a diploid) is likely to result in the establishment of a 44-karyotype. Diploid gametes (especially pollen) are also of frequent occurrence among the *Scilleae*. Even in the formation of the diploid gametes there can be seen an evidence of hybridity. It has been shown that the formation of these, is in some manner, due to the failure of cell wall development at some stage of meiosis of the pollen mother cells. Ellison (1937) believes that this failure to develop the particular cell walls is "possibly due to the hybrid nature of

the plants concerned, because this abnormality was not observed in any of the parental pure species“.

The 45-variety is presumably a horticultural species. Many such unbalanced karyotypes are reported in various horticultural plants. Satô (1938) has recorded a karyotype of *Amaryllis alba* with  $2n = 39$  and he thinks that it has been derived from hybridization. Similar cases of karyotype alteration have been recorded as due to elimination, fragmentation etc. The 46-type must have arisen by the operation of some of these processes.

That this species is a hybrid could also be inferred from the extremely irregular meiosis it exhibits. There is a varying degree of multivalent formation. Trivalent formation is very frequent, as also bivalent formation. Quadrivalents are occasionally formed. This may imply a certain amount of autosyndesis. In fact many of the irregularities usually found in an autotriploid are recorded in this species. Some irregularities almost characteristic of interspecific hybrids are also patent. It may therefore be regarded as a case of autopolyploidy and interspecific hybridization.

The absence of any known species of *Scilla* in India having diploid chromosome number of 16 and 20 makes one wonder if *Scilla indica* is an indigenous species at all. It is likely that while *Urginea indica* is a native of India, *Scilla indica* may be an exotic species. Further work on the other species *Scilla* also said to be indigenous to India, but which we have not been able to get, may throw light upon this question.

### Summary

Three karyotypes of *Scilla indica*,  $2n = 44, 45, 46$  have been recognised. The 45 type is 'broad-leaved', while the 44 and 46 are 'narrow leaved' and indistinguishable morphologically from one another.

The somatic chromosome complements have been analysed in the tree types.

Meiosis is extremely irregular and the irregularities have been described in detail.

Polyploid pollen formation has been recorded and this is related to the failure of cell wall development in the first division or in the two divisions.

The chromosomes in the diploid pollen grain exhibit what may be termed somatic pairing.

On the evidence of the various irregularities in meiosis and the analysis of the chromosome complements, it is suggested that *Scilla*

*indica* is a hybrid between a sixteen chromosomed species and a twenty-chromosomed species, the complement being suggested to be made up of a triploid set of the former and a diploid set of the latter.

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## Studies on the Chromosome Numbers in Higher Plants III \*

By

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*Received January 27, 1939*

### Materials

Materials used here were 42 species belonging to 22 families, raised from seeds. Some of them were sent from the chief botanical gardens in Europe, to the authorities of which the writer wishes to express his best thanks. The technique used in this work has been referred to in previous papers.

### The Mode of Cell Division in PMC

The plants described below all show the furrowing process in the mode of the partition wall formation of the pollen mother cells (cf. Sugiura 1936b).

### Numbers of Chromosomes

**Compositae:** *Cosmos sulphureus*, *C. diversifolius*.

The numbers of chromosomes formerly found in Coreopsidinae were in *Bidens* 12, 24, in *Coreopsis* 12, and in *Dahlia* 16, but none in *Cosmos*.

The writer formerly counted 24 somatic chromosomes in *C. bipinnatus* (1931). Now he has also counted 12 meiotic chromosomes in each of the above two species.

The *diversifolius* species has somewhat larger meiotic chromosomes than those of the *sulphureus* at the same stage, although the outer appearance of the former was much smaller. As there is no secondary pairing of chromosomes to be seen in the meiotic division in these species, the basic number of chromosomes in *Cosmos* is probably 12.

**Campanulaceae (Lobelioideae):** *Lobelia Cliffortiana*, *L. inflata*,  
*L. Richardsonii*, *L. tenuior*.

The reported chromosome numbers in the genus *Lobelia* are shown in the Table 1.

\* The studies were made under the subsidy from the Japan Society for the Promotion of Scientific Research.

Table 1

Plant names	n	2n	Authors
<i>Lobelia erinus</i>	8		Armand 1912
<i>urens</i>	14, 21	42×	de Vilmorin & Simonet 1927
	21		Okuno× 1937
	8		Armand 1912
<i>Dortmanna</i>	8		" "
<i>cardinalis</i>	7		de Vilmorin & Simonet 1927, Sugiura 1936
<i>Cliffortiana</i>	7		" " " 1937
<i>ramosa</i>	9		" " " 1936
<i>tupa</i>	21		" " "
<i>speciosa</i>	21		" " "
<i>syphilitica</i>	7×	14×	" " " Okuno× 1937
<i>inflata</i>	7×	14×	Okuno× 1937, Sugiura 1937
<i>dresidensis</i>	7	14	" " " "
<i>sessilifolia</i>	14×	28×	" " " "
<i>tenuior</i>	9		Sugiura 1937
<i>triquetra</i>	7×	42×	Okuno× 1937, Sugiura 1937
<i>Richardsonii</i>	21	42×	" " " "

The writer having already counted the meiotic numbers of chromosomes in three species, now studied the above four species, finding 7, 7 and 8, 9 and 21 meiotic chromosomes. It was very interesting to find these two meiotic numbers in *L. inflata*, namely 7 and 8. The latter number 8 has already been noticed. (Sugiura '37). In the second metaphasic figures there are 8, almost equal, chromosomes and 7, one of which a little larger, chromosomes in the same anther.

It may be conjectured that *L. erinus*, *urens* and *Dortmanna* may also have 8 chromosomes in addition to the normal 7 ones, judging from the above and from the results of Armand's investigation. In this case the 7 chromosome group is probably derived from the 8 chromosome group by fusing two of them. Multiple numbers of 8 and 9 have not been found yet, although those of 7 have already been found, namely 14 and 21. Thus we now conclude that 8 is the basic chromosome number in *Lobelia* and 7 and 9 are derived from it.

*Downingia pulchella*. The genus *Downingia* has not been studied karyologically yet. There are 11 meiotic chromosomes, one of which is very large like a nucleolus.

#### Rubiaceae: *Crucianella stylosa*.

The meiotic chromosome numbers in the genus *Crucianella* were ascertained by Lloyd (1920) to be about 10 in *C. macrostachya* and *gilanica*, by Fagerlind and Homeyer (1932, 34) to be 11 and 22 in other species. Thus there are two different basic numbers in the genus, namely 10 and 11.

We now know that the meiotic number of chromosomes in *C.*



*stylosa* is 11 and the former count should be corrected. Quite recently Fagerlind has counted 11 meiotic chromosomes in 9 species of *Crucianella* and 22 in another.

**Acanthaceae:** *Acanthus spinosus*, *Dicliptera resupinata*, *Ruellia amoena*, *R. dipteracanthus*.

The writer previously counted 28 meiotic chromosomes in *A. lusitanicus* and has now found 56 in *A. spinosus*. It is interesting to note that the outer appearances of *A. spinosa* is very much smaller than that of *A. lusitanicus* in spite of the fact that the chromosome number of the former is twice that of the latter. The pollen mother cells are very large, measuring about  $21.5 \mu$  in diameter.

*Dicliptera resupinata*. This has 20 meiotic chromosomes. The pollen mother cells are about  $15 \mu$  in diameter.

*Ruellia amoena* *R. dipteracanthus*. These two *Ruellia* both have 18 meiotic chromosomes. Combining the results of my former count with the present results, we now know that there are 16 and 18 meiotic chromosomes in *Ruellia*. Putting together these studies on *Acanthus*, *Ruellia*, *Thunbergia* etc., we are able to say that pollen mother cells of Acanthaceae are much larger than those of other families, while the chromosomes they contain are much smaller in comparison.

**Scrophulariaceae:** *Hebenstreitia dentata*.

*Hebenstreitia* belonging to Selagineae, has not been investigated karyologically yet. Formerly the writer found 7 meiotic chromosomes in *H. comosa* and recently also found the same number of chromosomes in *dentata*.

**Loganiaceae:** *Buddleia asiatica*, *B. curviflora* var. *venenifera*.

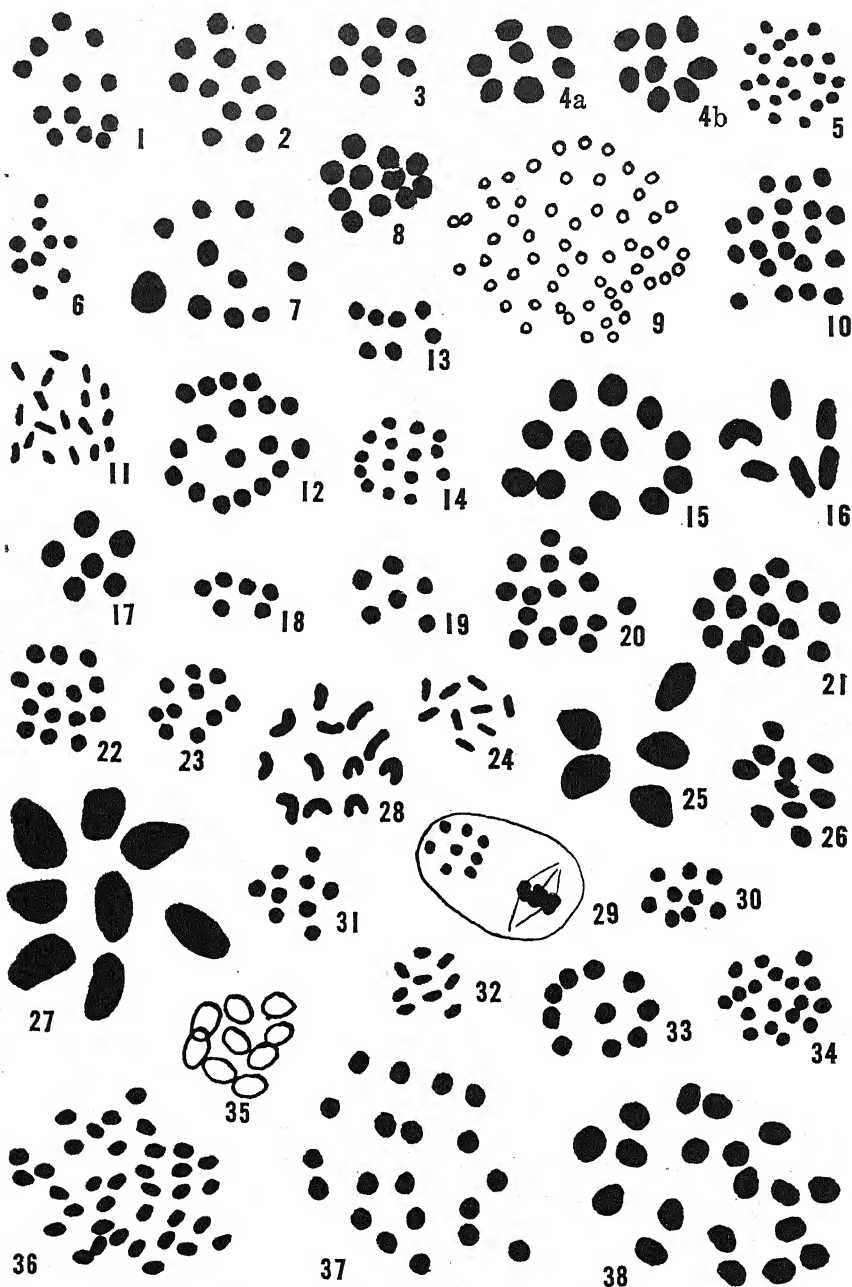
So far as the writer knows, the chromosome numbers in this family have not been ascertained. The present karyological investigation shows that the meiotic chromosome numbers in these species are all 15.

**Primulaceae:** *Lysimachia clethroides*.

Lewitsky has counted 24 somatic chromosomes in *Lysimachia vulgaris*, 34-36 in *L. nummularia*. The meiotic chromosomes counted here in the above species are 12. Quite recently Wulff counted 9 meiotic chromosomes in *L. nemorum*.

**Dispensiaceae:** *Dispensia obovata*.

Hagerup (1928) counted 6 meiotic chromosomes in *D. lapponica*. The writer also found the same number in this species.



Figs. 1-38.  $\times 3330$ . 1, *Cosmos sulphureus*. IM. 2, *C. diversiculatum*. IM. 3, *Lobelia Clifortiana*. IIM. 4a, b, *L. inflata*. IIM. 5, *L. Richardsonii*. IIM. 6, *L. tenuior*. IIM. 7, *Downingia pulchella*. IA. 8, *Crucianella stylosa*. IIM. 9, *Acanthus spinosus*. IM. 10, *Dicliptera resupinata*. IIM. 11, *Ruellia amoena*. IIM. 12, *R. dipteracanthus*. IA. 13, *Hebenstreitia dentata*. IA. 14, *Buddleia asiatica*. IM. 15, *Lysimachia clethroides*. IIM. 16, *Dispensia obovata*. IIM. 17, *Cuphea cyanea*. IM.

The second meiotic chromosomes are rod-shaped. The pollen mother cells are large in comparison with external appearances.

**Lythraceae:** *Cuphea cyanea*, *C. petiolata*, *C. lanceolata*.

So far as I am aware, the only chromosome numbers known in this family are those of the genus *Lythrum*. Former studies showed that the meiotic chromosome numbers in the *Lythrum* species were multiples of five.

In spite of that, *Cuphea* and *Lythrum* belong to the same section the Lythraee, the meiotic number of chromosomes of the former differing from that of the latter, the former being 6 while the latter 5, although multiples of 5 are only found. The same thing was also found in Heitz's *Antirrhinum* and also in my studies on *Armeria* and *Statice* etc.

**Loasaceae:** *Loasa hispida*, *L. triphylla*, *L. vulcanica*.

Formerly the writer counted 12 meiotic chromosomes in the pollen mother cells of *L. aurantiaca*. Three other species were examined, but these have different numbers of meiotic chromosomes; the former has 15, while the latter two has 14 each. Among the meiotic chromosomes of the *Loasa*, those of *L. triphylla* were the largest of all.

**Frankeniaceae:** *Frankenia pulverulenta*.

So far as I know there has been no karyological description of this family. The pollen mother cells are large in comparison with external appearances. There are found 10 meiotic chromosomes.

**Guttiferae:** *Hypericum polyphyllum*.

Nielson (1924) counted 9 meiotic chromosomes in this plant which has been confirmed by the writer.

In the pollen mother cell it was found that the first meiotic chromosomes were spherical while the second ones slender and rod-shaped like those of the somatic cells. The chromosome numbers hitherto counted in this genus are 7, 8, 9, 10, 16, 18 and 20 (cf. Winge 1925, Chattaway 1926).

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18, *C. petiolata*. IIM. 19, *C. lanceolata*. IA. 20, *Loasa hispida*. IM. 21, *L. triphylla*. IM. 22, *L. vulcanica*. IM. 23, *Frankenia pulverulenta*. IM. 24, *Hypericum polyphyllum*. IIM. 25, *Limnanthus alba*. IM. 26, *Cleome trachysperma*. IA. 27, *Cimicifuga acerina* var. *obtusiloba*. IA. 28, *Lychnis chalcidonica*. IIM. 29, *Corrigiola littoralis*. IIM. 30, *Delosperma herbeum*. IIM. 31, *D. Steytlerae*. IA. 32, *Mesembrianthemum multiflorum*. IIM. 33, *Emex spinosa*. IM. 34, *Phytolacca octandra*. IIM. 35, *Ph. sessiliflora*. IIM. 36, *Petiveria alliacea*. IIM. 37, *Cymbidium sinense*. IM. 38, *Aerides japonicum*. IM.

I believe that 4 is the probable basic number in this genus as Winge stated (1925).

**Limnanthaceae:** *Limnanthes alba*.

The writer previously counted 5 meiotic chromosomes in *L. Douglasii*, and recently also found the same number in the above species. Their size and shape are very similar to those of the former.

**Capparidaceae:** *Cleome trachysperma*.

It has 10 meiotic chromosomes. As to the chromosome numbers in the genus *Cleome* it has been known that they vary; viz. 10, 11, 12, 16, 19, 70, and 140. Even in the same species, for example, *C. spinosa*, three numbers have been found, 10, 12, and 19. As it is, the basic number cannot be determined at present.

**Ranunculaceae:** *Cimicifuga acerina* var. *obtusiloba*.

The genus *Cimicifuga* has been studied karyologically by Lewitsky, Nakajima and Langlet. These writers, however, have only studied somatic chromosomes, their counts being 16. The writer studying the pollen mother cells, found 8 chromosomes which were as large as those of *Adonis* (Sugiura 1936).

**Caryophyllaceae:** *Lichnis chalcedonica*.

Blackburn (1928) and Sokolowa (1931) have studied this and counted 12 meiotic chromosomes. My own count was the same. The second meiotic chromosomes are V shaped.

*Corrigiola littoralis*. Rocén (1927) counted about 8 meiotic chromosomes in this species and Blackburn (1934) 18 somatic. The writer, however, has counted 8 meiotic ones which are very small together with the pollen mother cells.

**Aizoaceae:** *Delosperma herbeum*, *P. multiflorum*, *P. Steytlerae*.

There have been no karyological studies on the genus *Delosperma* yet.

They have 9 spherical chromosomes each. The meiotic chromosomes are generally much smaller than those of *Mesembryanthemum*.

**Polygonaceae:** *Emex spinosa*.

This plant has been already studied by Jaretsky (1927) and Edman (1929), both having counted 10 meiotic chromosomes. The writer's count was the same.

**Phytolaccaceae:** *Phytolacca octandra*, *P. sessiliflora*.

We have studied the above two plants and found 18 meiotic chromosomes in the former and 9 in the latter. The latter plant

Table 2

Plants investigated	n	IM ( $\mu$ )	IA ( $\mu$ )	IIM ( $\mu$ )	IIA ( $\mu$ )	Fig.
Compositae						
<i>Cosmos sulphureus</i>	12	0.68				1
<i>C. diversiculatum</i>	12	0.75				2
Campanulaceae						
<i>Lobelia Cliffortiana</i>	7			0.93		3
<i>L. inflata</i>	7, (8)			$1 \times 0.7$		4a, b
<i>L. Richardsonii</i>	21			0.48		5
<i>L. tenuior</i>	9			0.6		6
<i>Downingia pulchella</i>	11			$0.6-1.4$		7
Rubiaceae						
<i>Crucianella stylosa</i>	11			0.72		8
Acanthaceae						
<i>Acanthus spinosus</i>	56	0.5				9
<i>Decliptera resupinata</i>	20			0.62		10
<i>Ruellia amoena</i>	18			$0.68 \times 0.37$		11
<i>R. dipteracanthus</i>	18		0.68			12
Scrophulariaceae						
<i>Hebenstreitia dentata</i>	7		0.6			13
Loganiaceae						
<i>Buddleia asiatica</i>	15	0.5				14
Primulaceae						
<i>Lysimachia clethroides</i>	12			1.25		15
Dispensiaceae						
<i>Dispensia obovata</i>	6			$1.4 \times 0.6$		16
Lythraceae						
<i>Cuphea cyanea</i>	6	1.0				17
<i>C. petiolata</i>	6				0.55	18
<i>C. lanceolata</i>	6		0.75			19
Loasaceae						
<i>Loasa hispida</i>	15	0.75				20
<i>L. triphylla</i>	14	1.00				21
<i>L. vulcanica</i>	14	0.68				22
Frankeniaceae						
<i>Frankenia pulverulenta</i>	10	0.68				23
Guttiferae						
<i>Hypericum polyphyllum</i>	9		0.5			24
Limnanthaceae						
<i>Limnantes alba</i>	5	1.5				25
Capparidaceae						
<i>Cleome trachysperma</i>	10		$0.75 \times 1.0$			26
Ranunculaceae						
<i>Cimicifuga acerina</i> v. <i>obtusiloba</i>	8		$2.5 \times 1.5$			27
Caryophyllaceae						
<i>Lychnis chalcidonica</i>	12			$1.1 \times 0.3$		28
<i>Corrigiola littoralis</i>	8			0.5		29
Aizoaceae						
<i>Delosperma Steylerae</i>	9		0.7			30
<i>D. herbeum</i>	9					31
<i>Mesembryanthemum multiflorum</i>	9			$0.7 \times 0.4$		32
Polygonaceae						
<i>Emex spinosa</i>	10	0.75				33
Phytolaccaceae						
<i>Phytolacca octandra</i>	18			0.6		34
<i>P. sessiliflora</i>	9		$1.2 \times 0.75$			35
<i>Petiveria alliacea</i>	36	$0.75 \times 0.6$				36
Orchadaceae						
<i>Cymbidium sinense</i>	20	0.75				37
<i>Aerides japonicum</i>	19					38

being as tall as *P. acinosa*, the meiotic chromosomes are twice as large as the former, the epidermal cells of the leaves in the latter are also larger than those of *Phytolacca decandra* ( $n = 18$ ). The basic chromosome number in the genus *Phytolacca* has therefore been definitely determined to be 9.

*Petiveria alliacea*. There have been no karyological studies on this genus. The meiotic chromosomes, being 36 in number, are rather big in spite of the large number of chromosomes.

**Orchidaceae: *Cymbidium sinense*.**

Suessenguth (1920) counted 9–10 meiotic chromosomes in *C. Lowianum* but afterwards Hoffmann (1920–30) found 20 in the same plant.

*Aerides japonicum*. No karyological descriptions of the chromosome numbers are known for *Aerides*. We have now found 19 meiotic chromosomes in this species, while *Sarcanthus rostratus*, belonging also to the Sarcanteae, has 18 meiotic chromosomes.

The results of the present investigation are summarized in Table 2.

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## Chromosome Structure A critical review \*

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### Contents

	Page
Introduction . . . . .	213
I. Chromosomes in mitosis . . . . .	217
II. Chromosomes in meiosis . . . . .	225
III. Apparent changes in chromosome structure as related to water-content . . . . .	234
IV. Chromosome cycle. . . . .	241
V. Conclusion . . . . .	250

### Introduction

The internal structure of the chromosomes was considered in the very early days of chromosome study, and many opinions have since been put forward on the problem. They may be classified into two groups: those that consider the chromosomes to be of an optically homogeneous structure and those that consider them as being of some heterogeneous structure (SAKAMURA, 1927). Of these different opinions, the opinion that the chromosome consists of a linear series of chromomeres had at one time received a general acceptance, especially of those engaged in chromosome study with fixed material. In the early days, another structure was also ascribed to the chromosome—the spiral structure of BARANETZKY (1880), but this structure was regarded by STRASBURGER (1884) who followed the view of BALBIANI and PFITZNER as no more than the chromomere structure. It seems that in those days the view of the chromomere structure had been greatly influenced by the study of BALBIANI on the salivary nucleus of the *Chironomus* larvae. It has also received until recently some theoretical support: first, in the fact that from the view point of the chromosomes as carriers of the hereditary units, a discontinuous structure is more plausible than the continuous one; and second, that this structure can fulfill the requirement (also demanded by that point of view) that the longitudinal division of the chromosomes must be a meristic one (comp. WILSON, 1925). The spiral structure view, on the other hand, notwithstanding the fact that this structure was later repeatedly observed by several investigators, and by VEJDOVSKÝ the term "chromonema" was introduced for the spiral thread, had received little attention of the investigators, in view of the fact that if the spiral is an important component of the chromosome as are the chromomeres, the longitudinal division of the chromosome (VEJDOVSKÝ,

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1911-12; MARTENS, 1922) does not result in the meristic division or the quantitative reduction of each unit structure duplicated by division, but in a qualitative reduction in which every other half turn of the spiral is distributed into the different daughter chromosomes (comp. WILSON, 1925). Meanwhile other views appeared, such as those of the cylindrical structure and those of the vacuolated structure. When, however, in 1926, KAUFMANN asserted the spiral structure in his study with *Tradescantia*, the classical material with which BARANETZKY first observed this structure in pollen mother cells, many papers appeared in rapid succession confirming that this is the true structure, and to-day when little more than a decade has elapsed, many important results, the literature on which is referred to in STRAUB's "Sammelreferat" (1938), are accumulating. Why has the formerly so neglected theory of the spiral structure suddenly come to have such a keen interest for investigators? The reason would probably lie in the recent development in the study of genetics. SEIFRIZ (1930), who mentions CHAMBERLAIN's statement thus: "any theory of heredity which is based on a linear arrangement of genes will have to be abandoned if it cannot be reconciled with a vacuolated structure of the chromosomes", says: "the geneticists have, unwittingly, now satisfied CHAMBERLAIN's request by converting the straight gene string into spiral".

The complete solution of the problem is of course complex, but even in the present state of our knowledge the theory of the spiral structure distinguishes itself from the other theories in the following two points:—

1) It is to-day an established fact that the chromosome changes its form during its mitotic cycle. In all the theories we have, other than the spiral theory as it is understood at the present day, there is no direct morphological connection in the structure between one stage and another and the form change has been interpreted by postulations. The form change from metaphase to the resting stage, for instance, is interpreted by the assumption of alveolation in one theory, and by spiral differentiation in another. In the spiral or chromonema theory of the present day, on the other hand, there is in the origin of the chromonema no differentiation from an undifferentiated substratum—a chromonema is brought forth from a chromonema by division, and there is a direct morphological connection between the two stages, metaphase and resting stage. The chromonema exists in both stages, the only difference between them is the difference in the manner of behaviour. The change is continuous. In general, both continuous and discontinuous changes may take place, but when a question concerns such a problem as the chromosome individuality a continuous change seems more reasonable for the interpretation than a discontinuous change.

2) Any theory rests on observed facts which involve each a truth, but if the facts used are limited and only sufficient to indicate one side of a phenomenon, theories thus postulated will be different. If, in this case, the whole facts could be considered, the different theories would be brought into harmony. If, therefore, a theory can bring other theories into harmony, it must be one worth using at least as a working hypothesis



for the further study intended to reveal the true nature of the phenomenon. There is a high probability that the spiral theory will prove to be such a theory as can harmonize all the theories of the chromosome structure hitherto put forward (cf. FUJII, 1926).

When these points are considered, we see that the spiral theory is most important among the theories of the chromosome structure, and in this sense the spiral structure mainly will be considered in the present review.

In the theory of spiral structure, we find again different views on each question. They have been recently reviewed throughout and criticized by STRAUB (1938) in his "Sammelreferat". In the present paper, accordingly, the problem is considered from one single point of view, and an effort of trying to harmonize these different views is attempted.

Before entering the main part of this paper, it would be convenient briefly to consider the types of the spiral<sup>1)</sup> (Fig. 1).

As to the manner of formation of a regular spiral, we may consider two cases (DARLINGTON, 1935 a): 1) the formation by rotations of a thread with a free end (comp. case 1, KUWADA, 1927). 2) the formation in a thread with both ends fixed (comp. case 2 of the same). In the first case the thread itself is not twisted at all through its whole length. This is the ordinary spiral and may be called orthospiral. In the second case the thread is twisted in the opposite direction to the spiral, as many times as the number of the spiral turns. When the twists are uniformly distributed throughout the spiral, this regular spiral may be called anorthospiral. When an orthospiral is drawn out, the thread becomes twisted, and if its end or ends are free it rotates to untwist. In the case of the anorthospiral, the twisting is cancelled out by the twists of the thread, so that no rotation is brought about. Both orthospiral and anorthospiral may be single—a single spiral, or double—a double spiral. In the case of the orthospiral, a double spiral can not be separated into two independent spirals, the component spirals intertwining with each other on every level of the turn. In the anorthospirals, on the other hand, a double spiral can be separated freely into the component spirals without their intertwining. The former is called a double-stranded spiral and the latter a compound spiral. If an orthospiral is divided into two daughter spirals by the splitting of the thread carried out along its axis, it becomes a double stranded spiral. If an anorthospiral is divided in the same way a compound spiral is formed. Two orthospirals of different origin may also form a compound spiral by appression into each other if the direction of coiling is the same. In the double-stranded spiral we have three forms according to the degree of approximation of the two threads. 1) The double-stranded spiral with its strands united apparently into one or closely approximated to each other—a united double-stranded spiral or an approximated double-stranded spiral. 2)

1) What we call here "spiral" is strictly speaking a cylindrical spiral, but for the sake of convenience it is referred to simply as a spiral.

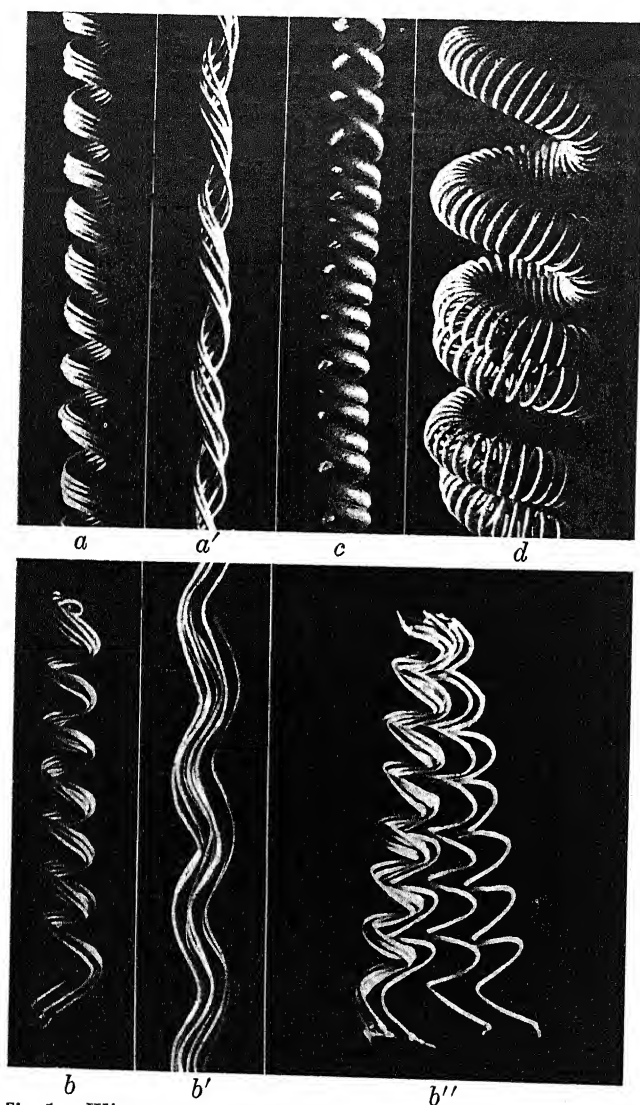


Fig. 1. Wire model showing types of spirals. *a* and *b*. Spirals made of a bundle of wire strands. *a*, orthospiral; *b*, anorthospiral. In *b* the component spirals of single strand are quite independent from one another and freely separable (*b'*), showing that in the case of the anorthospiral the two spirals produced by any cleavage along the length of the strands composing the spiral are separable. In *a* these spirals of single strand are relational to one another and not separable completely (comp. *a'* and *c*). When the spiral is drawn out, the wire strands of the anorthospiral run parallel (*b'*), while those of the orthospiral are twisted (*a'*). *c*. Double stranded spiral showing different configurations in different parts: "relational spiral" in the upper, "spiral with apparent pitch" in the middle, and "approximated spiral" ("united spiral") in the lower. *d*. A double coiled spiral in which the major spiral is double stranded and the minor spirals form a compound spiral in the upper half of the major spiral.

The double-stranded spiral with its strands separated from each other so as to present an appearance at first sight of a single spiral with a small pitch—a double-stranded spiral with apparent or divided pitch. 3) The double-stranded spiral with its strands separated from each other so as to present the form of the two intertwining spirals or that of the pro-phasic chromatids twisted around each other—a relational spiral, a term borrowed from DARLINGTON.

A double-stranded spiral can easily take any of these three forms by a simple change in degree of approximation of the two strands, so that different parts of a double-stranded spiral can present different forms of the spiral. The compound spiral has no variety. If, in the latter, the two component spirals are coiled in opposite directions, it may appear at first sight to be a relational spiral seen from one angle and a double

spiral when seen from another angle perpendicular to the first, but parts of different levels can not present different appearances; the whole spiral can have only one appearance seen from any one angle. In the compound spirals, therefore, the special relation between the two strands is always fixed, not changeable. This is the point which distinguishes clearly between the two spirals, the double-stranded and the compound. An orthospiral can be a double-stranded spiral or a compound spiral, but the anorthospiral can not be a double-stranded spiral. Whether a double spiral is of the double-stranded type or not is an important criterion for the determination of whether it is an orthospiral or an anorthospiral.

One spiral can be coiled inside another larger spiral. This double-coiled spiral may also be a double spiral, and both double-stranded and compound types exist. In the former case, only one spiral, either the major spiral or the minor can be the double-stranded spiral, while in the latter both can be of the compound type at the same time.

### I. Chromosomes in Mitosis

At the maximum development stage in metaphase and anaphase, the chromosomes of large size show the spiral structure (comp. Fig. 2) in which the chromonema or chromonemata are coiled more or less regularly (KUWADA, 1926; GEITLER, 1935, 1938). This is, however, not always the case. It depends upon the method of fixation or the treatment of the chromosomes whether this structure is visible or not. In most fixed material, the chromosomes appear to be homogeneous in structure, the whole body being stained solid. Beside this solid structure, the chromomere structure (STRASBURGER, 1884; NAWASHIN, 1910; cf. MIYAKE, 1905), the cylindrical structure (BONNEVIE, 1908; LUNDEGÅRDH, 1912 *a*; SCHUSTOW, 1913; CHAMBERS, 1924) and the vacuolated structure (OVERTON, 1922; CHAMBERLAIN, 1925) have also been observed. When, however, the telophase is reached, some structure becomes visible in all cases, and often even in the late anaphase. In these stages the chromosomes appear to show sometimes an axial alveolation and sometimes a zig-zag or a spiral structure within. While MERRIMAN (1904) has interpreted a similar structure which she observed in the anaphase to be a tubular structure transformed from the chromomere structure in earlier stages, LUNDEGÅRDH (1910, 1912), FRASER and SNELL (1911), DIGBY (1910, 1919), FRASER (1914) and others have regarded it as representing the longitudinal split of the chromosome. The latter view was criticized by



Fig. 2. Chromosomes in homotype division in *Tradescantia reflexa* showing the thread nature of the chromonemata in spiral (the lower chromosomes unravelled in the middle of the figure). Treated with N/100  $\text{NH}_4\text{OH}$  and stained with acetocarmine. (After MIMURA, unpublished).

some authors, especially by SHARP (1913) who reached the conclusion that the alveolation in the telophase has "nothing whatever to do with the longitudinal splitting of the chromosomes", but shows the transition from the solid structure in metaphase and anaphase to the reticulate structure of the resting nucleus. SHARP (1929), re-examining later the chromosomes in the root-tips of *Vicia* and some other plants from the view point of the spiral structure, reached another conclusion, however:—"Now that we know the chromonemata to be present even in the anaphase, it is evident that the structure observed within telophase chromosomes is not actually produced at this time by alveolation or any other process, but is simply rendered visible through a reduction in the chromaticity of one of the constituents of the chromosome". He has thus abandoned the term "alveolation", but regards "the anastomoses which join the chromonemata of neighbouring chromosomes to form the interphasic reticulum" as the only new elements that are conspicuous in the interphase. In his observation with living material, TELEŻYŃSKI (1930) also believes in the existence of the "anastomoses" between chromosomes, but NAITHANI (1937) and others regard it as a mere artifact.

In the interphase and resting stage, the structure of the nucleus appears to be very complicated. The more rigid component of the structure is generally called "reticulum". The formation of the reticulum from the chromosomes was interpreted by GRÉGOIRE and his school by alveolation and by BONNEVIE (1908) by spiral differentiation in the chromosomes. In both these interpretations, there is a discontinuity in the transformation, or the rise of a new phenomenon, the alveolation or the spiral differentiation. If the chromosomes are, on the other hand, of the spiral or the thread structure, the reticulum would be interpreted as formed of the threads of the chromonemata through a slight change in form of their spiral without any discontinuity in the transformation. Careful observations of both fixed and living materials seem to show that the reticulum is not a network structure with chromatin granules on the knots as assumed by the earlier workers, but a thread structure (BĚLAŘ, 1929; SHIWAGO, 1926; LEE, 1921; KAUFMANN, 1926; NEBEL, 1932; FUJII and YASUI, 1935; and others). BĚLAŘ (1929) has confirmed this theory of the thread nature of the reticulum by a careful manipulation of the microscrew of the microscope, and SHIWAGO (1926) by observation of the movement of the structure in living nuclei. The results obtained by recent workers are converging on the view that in the interphase and resting stage, the chromonemata are rendered more or less loosely or even irregularly coiled so that the chromosome territories become hardly distinguishable from one another, thus forming a structure of the complex appearance, the reticulum, but still retaining their spiral character (NEBEL and RUTTLE, 1936).

When the nucleus enters the prophase, a change takes place in the nucleus through which it comes to present an aspect which is very similar to the structure in the late telophase. SHARP (1929) describes it thus: "As a result of the disappearance of anastomoses and the increasing regularity in the arrangement of the chromatic matter, the conspicuous

'spiral stage' of the early prophase is reached" (p. 367). BĚLAŘ (1929) has observed this stage in living staminate hair cells of *Tradescantia virginica*. He considers that the "früheste Stadium" is represented by his Text-fig. 26 (from fixed material) which he identifies, as is clear from his description, with his photomicrograph (from living material) reproduced in his Plate-fig. 3, which he explains as the "Stadium der Chromosomenspiralen". KUWADA and NAKAMURA (1934b) have traced the chromosome development in single living cells in the staminate hairs of *Tradescantia reflexa* from this stage of the "Chromosomenspiralen" of BĚLAŘ or the "spiral stage" of SHARP up to the metaphase in one case, and to the interphase in another (cf. KUWADA, SINKE and NAKAZAWA, 1939). NAKAZAWA (KUWADA, SINKE and NAKAZAWA, 1939) has also traced this development in single living cells of the same staminate hairs from the very beginning of the prophase where the nucleus presents practically the usual 'reticulate' appearance up to the close of mitosis, and has confirmed that the spiral stage is the earliest stage the existence of which we can recognize free from obstruction. In fixed material the nuclei in the spiral stage appear very similar in structure to those in the late telophase. "Die Kerne sehen auf diesem Stadium späteren Telophasekernen oft zum verwechseln ähnlich" (BĚLAŘ, 1929, p. 78). From the results of observation of living cells in mitosis, it is clear, however, that there is no room for doubting the real existence of the spiral stage in the prophase. It can emphatically be said so far as the staminate hairs of *Tradescantia* are concerned, that the spiral stage exists in the early prophase. It may be a question, on the other hand, whether this spiral form is of universal occurrence in prophase or not, but it seems likely that it exists in a certain form (KUWADA, SINKE and NAKAZAWA, 1939).

DARLINGTON (1935) has described the change in the beginning of the prophase which he observed in *Fritillaria* somewhat differently from that mentioned above. According to him, in the late telophase the spiral of the earlier stage becomes larger in diameter by the relaxation of a "minimum spiral" which cannot be directly observed, causing the distortion of the whole system into a super-spiral. This change takes place in the telophase, but it does not reach its maximum extent until the ensuing prophase. In the resting stage apparently no further changes take place in the form of the spiral. At first when the prophase begins, the super-spirals become more strongly developed, and thus the straight general arrangement of the chromosomes which is characteristic of the early telophase is lost. In this interpretation of the prophasic change the spiral stage is omitted. DARLINGTON (1935) is of the opinion that the drawing of prophase given by SHARP which we call the spiral stage is really of telophase. It is a matter of question whether or not the spiral stage is characterized by the general straight arrangement of chromosomes (KUWADA, SINKE and NAKAZAWA, 1939), but it seems highly probable that in *Fritillaria* too, a certain stage which corresponds to the spiral stage may exist. Recently it has been found that in the preleptotene stage there is a stage very similar to the spiral stage, where it had been

overlooked in plant meiosis ever since the investigation of meiosis was begun (NEBEL and RUTTLE, 1936; HIRAOKA, p. 1.<sup>1)</sup>).

After the spiral stage, the spirals tend to be drawn out, being associated with the thickening of the threads, and perhaps this thickening may play some important rôle in the mechanism of drawing out (KUWADA and NAKAMURA, 1934b; SAX and SAX, 1935; GEITLER, 1935). The drawn out threads show "relic coils". They are found later to be longitudinally double. The double thread or pair of sister chromatids are intertwined with each other to some variable extent (KOSHY, 1933), forming the "relational spiral" of DARLINGTON (1935). The assumption that the intertwining between the chromatids is due to uncoiling of the old spirals is possible only when the spirals are orthospirals (cf. SAX, 1936). An-orthospirals do not rotate when they are drawn out, and consequently no intertwining results between them. The chromatids gradually thicken in diameter and contract in length, ultimately to form the metaphase chromosomes.

The expansion in diameter and the contraction in length of the chromatids is accompanied by a new spiralization of the chromonemata (KUWADA and NAKAMURA, 1934b; DARLINGTON, 1935; SAX and SAX, 1935; GEITLER, 1935; NEBEL and RUTTLE, 1936). The process of drawing out associated with the formation of the new coils in the chromatids is clearly illustrated in BĚLAŘ's Text-fig. 2b-e (KUWADA and NAKAMURA, 1934b).

The new coils which are perceivable from BĚLAŘ's Text-figures are usually not directly visible (DARLINGTON, 1935). Just recently, AISIMA (p.l.) succeeded in observing them directly with his maceration method. According to him the spirals in the spiral stage are of the double-coiled structure which is comparable with the meiotic spirals in *Tradescantia* (Fig. 3). From this result of AISIMA's investigation, it could be concluded that the new coiling has begun already in the spiral stage as seems likely if we regard the drawing out of the old spiral as being connected with the new spiralization. STRAUB's "Spiralprophase", where the new spirals are visible, now becomes one with the "spiral stage" where the old spirals are distinct; both terms would be used for the same stage.

From what we have said above, it may be seen that the spiral theory explains the individuality of chromosomes, which is the most important problem in dealing with the chromosome structure, more adequately than the other theories can do, if it is assumed that the spiral thread or the chromonema is the essential part of the chromosome, because in this theory the chromonema exists throughout the stages of the chromosome cycle, the apparent changes in chromosome structure being only the changes in the form of the spiral which the chromonema assumes.

In the drawing of SMITH (1932), we find some important facts which require discussion. In Fig. 2 (Pl. I) of SMITH which is reproduced in our Fig. 4, for instance, the spiralized chromonema appears to be single in both chromosomes on the left and in the middle of the Figure,

1) p.l. = To be published later.

except for the end gyre which is clearly double. In the chromosome on the right hand side the spiral is single-stranded in the lower half and a relational spiral in the upper. If we assume that in these chromosomes a double-stranded spiral is contained, we can readily understand the reason why a part of the spiral appears sometimes to be single-stranded

and sometimes double-stranded or a relational spiral, because the double-stranded spiral can assume any of these forms by a simple change in degree of approximation of the two strands. In many other figures of

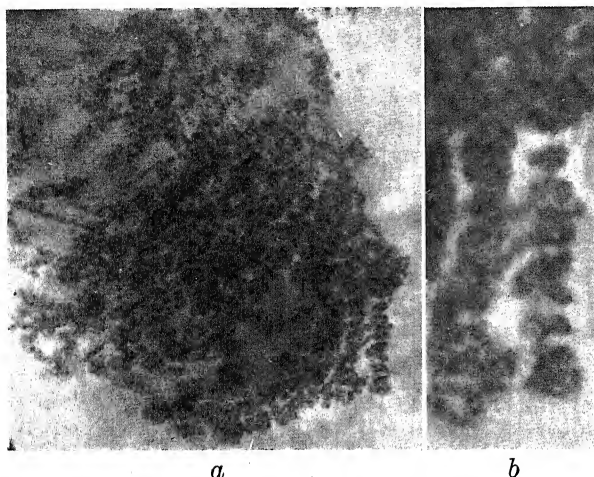


Fig. 3. Nucleus in spiral stage from *Tradescantia* root-tip macerated and stained with acetocarmine, showing minute new coils of the chromonemata. The nucleus is crushed by pressing. *b* is an enlargement of two spirals in the right lower corner of *a*. (After AISIMA).



Fig. 4. Anaphase chromosomes in root tip of *Galtonia*. (After SMITH).

SMITH we also see the same aspect of the double-stranded spiral with its appearances different in different parts. These partial changes in appearance of a spiral can not be realized in the cases of other types of the spiral. The conclusion is then drawn that in the *Galtonia* studies by SMITH the spiral of the chromonemata must be the double-stranded spiral. Two questions then arise:

1) In SMITH's Fig. 2 in which anaphasic chromosomes are shown (Fig. 4), the number of spiral turns is much smaller, the chromosomes being of the meiotic form, than we expect from the number (20–25) given by SAX and SAX (1935) for the somatic chromosomes of *Tradescantia* (comp. also UPCOTT, 1935). 2) In the same figure of SMITH, the number of the turns of different chromosomes varies to so great an extent that one can hardly imagine so much variety in chromosomes of a similar size and in the same stage.

A thorough explanation of these questions seems to be rather difficult, but the result obtained by AISIMA (p.l.) in his observation of the root-tip chromosomes of *Tradescantia reflexa* with his maceration method affords a valuable suggestion for their solution. His result shows that in the anaphase the two sister chromatids, each of the spiral structure, are found twisted around each other. The figures of the chromosomes are



very much complicated by the spiral turns contained in each chromatid, and it is not possible exactly to determine in each chromosome in what degree the chromatids are twisted, but, by inference from some clear figures such as that shown in Fig. 5, *b*, it is beyond question that the number of twists is much less than the

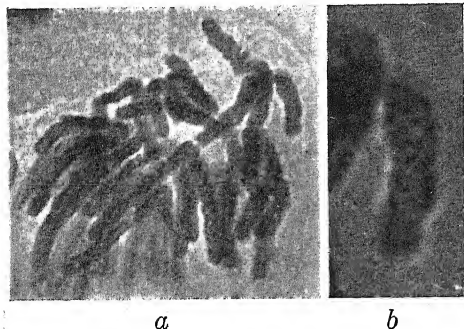


Fig. 5. An anaphase chromosome group from *Tradescantia* root tip. *b* is an enlargement of the chromosome in the upper right corner of *a*, showing twisting between chromatids.

number of spiral turns contained in the chromatids, and it seems also that the degree of twisting varies in different chromosomes to a considerable extent. The twisted aspect is the characteristic feature of the relational spiral or, speaking more generally, that of a drawn out double-stranded spiral. The anaphasic chromosomes observed by AISIMA are, therefore, regarded as double-coiled spirals with drawn out double-stranded major spirals. In fixed material it is common occurrence for a double-coiled spiral to appear to be a single-coiled spiral, the minor spiral being

concealed as the effect of fixation. If, therefore, it is assumable that the chromosomes in SMITH's figures are those in which the minor spirals are concealed, and that only the major spirals or the chromatid spirals which are here in the contracted form are visible, then the chromosomes become comparable with those of *Tradescantia* studied by AISIMA.

A new question arises, then, how in the anaphase the two chromatids each containing an independent spiral can become twisted around each other or even double-coiled, a question which should be considered first. As mentioned in the end part of the introduction, if a spiral is an orthospiral, the daughter spirals produced by splitting it along the longitudinal axis of its thread form a double-stranded spiral, and if it is the anorthospiral, the daughter spirals thus formed are two free spirals. The two chromatids in the anaphase chromosome must be derived from the mother chromosome by a longitudinal halving. We have here to assume that this halving should originally take place along the long axis of the chromonema in the chromosome, because the chromonema is the only component of the chromosome which strictly maintains its individuality throughout the chromosome cycle. In the somatic phase the chromonemata are coiled, practically through all stages of the cycle. If, therefore, these chromonemata are longitudinally halved, the daughter chromonemata or spirals produced will be freely separable or will not be, according to the type, orthospiral or anorthospiral, to which the chromonema spiral belongs. We may then find the reason why the two chromatids form a double-stranded spiral (*Galtonia*) or become twisted around each other (*Tradescantia*) in the anaphase. The reason



is simply this: the chromonema spiral is an orthospiral. This exclusively orthospiral nature of the chromonema spiral being assumed, we shall next look into the form changes of the chromonemata taking place in the chromosome cycle.

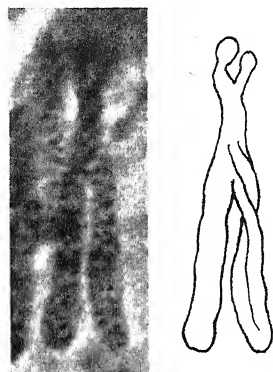
It now seems highly probable that the anaphase spirals of *Galtonia* which SMITH has observed and the anaphasic chromatid twists of *Tradescantia* observed by AISIMA are the relics of the "old" spirals remaining not completely drawn out in the prophase, because in the latter new spirals are observed to be contained in the chromatids. The first question which we raised above, about the reduction in the number of the spiral turns, may then be explained as being due to the prophasic thickening of the spiral threads or their new spiralization. The reduction may proceed to a variable extent in connection with other factors, so that the second question as to why the number of the spiral turns may be different in different cases could be understood. According to the state of contraction to which the anaphasic chromosomes are subjected, the two types of *Galtonia* and *Tradescantia* will result. In both cases, the spiral in each anaphasic chromatid coils or should coil, (if it really does coil), quite independently from the other. This fact shows that in the spiralization in the prophase the two chromonemata which coil must have been quite free from each other. They had, however, as sister chromonemata, formed together previously a double-stranded spiral which was subsequently straightened in connection with the new spiralization; and consequently after the contraction the half-chromatids containing the new spirals or the chromatids in the anaphase appear as we see them in SMITH's or AISIMA's figure. In *Galtonia* the relic or chromatid spirals (major spirals) in the anaphase reappear in the next early prophase but then disappear being drawn out.

While in SMITH's drawings the major spirals are clearly shown, in others, they are obscure. In these cases only the new spirals are conspicuous, and the number of the gyres is considerable. This condition has been expressed by WADA (1933) in saying that "die Spiralwindung der somatischen Chromosomen auffallend eng anliegt" (cf. UPCOTT, 1935; GEITLER, 1935). It seems highly probable that in these cases too, as in *Tradescantia* studied by AISIMA, the two chromatids are twisted around to some slight extent and are in close contact with each other. This condition is also shown by the I-anaphasic chromosomes of single-coiled structure in Fig. 6 reproduced from the photomicrograph of KATO and IWATA (1935), though at first sight the two spirals appear to be a single spiral.

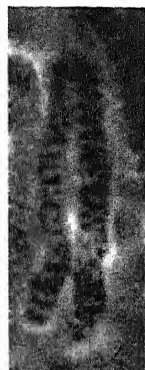
In both Figs. 5 and 6, the two spirals in the anaphasic chromosome look as if they are each furnished with their own matrices, and thus we described them above as chromatids. The two chromatids may be invested together with one common matrix, the chromosome matrix. One might expect that the different behaviour of the matrices of different kinds such as the chromosome matrix and the chromatid matrix would cause the chromosome to present an internal structure apparently variable (see Section IV).

It has been reported that in some plants the chromosomes are considerably shorter in the pollen grain division than in the root tip division. SAX and SAX (1935) have given the following numerical results:—

	Root tip	I-division	Pollen grain
<i>Vicia faba</i>	13	9	11
<i>Tradescantia</i> sp.	21	9	12
<i>Lilium regale</i>	22	12	15



a



b

Fig. 6. Chromosomes in I-anaphase of *Lilium* pollen mother cell, showing twisting between half-chromatids. Enlargements from the original photomicrograph. The accompanying diagrams are drawn by KATO. (After KATO and IWATA).

The chromosomes are in a more contracted form when they are short than when longer. It seems thus highly probable that when short they are of a double-coiled structure as in *Galtonia*. The result obtained by KUWADA and NAKAMURA (1934 c) by means of the polarization microscope is in accord with this view (cf. GEITLER, 1935). The result shows that in *Tradescantia reflexa* the sign of the double refraction presented by the pollen grain chromosomes is the same in a high frequency as that of the meiotic chromosomes of the double-coiled structure.

In *Galtonia* two "chromonemata" are reported to have been observed by SMITH in the chromosome. While many authors maintain this view that there are two chromonemata, some others are of different opinions. DARLINGTON is of the opinion that a single chromonema is contained in the chromosome, and NEBEL, four in a single chromosome. It is indeed a very difficult task accurately to determine how many chromonemata are contained, but the fact that the anaphasic chromosome contains two chromatids of spiral structure should not be over-looked in consideration of the question, because observation should be more accurate when the chromatid is double than when the chromonema is double. It is true that every opinion rests on observation. It seems then safe to assume that the number of chromonemata in the chromosome is four, and we

may bring the three opinions into harmony by the assumption that under certain circumstances the four chromonemata appear by union to be two or even one. There seems to be a good possibility of such an apparent reduction of the number by union (cf. NEBEL and RUTTLE, 1936). This will be considered in Section III.

While in fixed material, as mentioned above, if fixation is adequate, the chromonemata are visible throughout the stage in the chromosome cycle, in living material they are visible only in certain limited stages,

if the cell is quite healthy. BĚLAŘ (1929) has shown that in the staminate hairs of *Tradescantia* the chromonemata are visible, in the living state, in the late telophase, interphase and the early prophase, but not in the late prophase, metaphase and anaphase. KUWADA and NAKAMURA (1934 b) have obtained the same result with living staminate hair cells mounted with liquid paraffin which TELEŻYŃSKI (1930) used with successful results in his investigation. This result explains the reason why in fixed material the chromosome appears often to be solid in metaphase and anaphase while in telophase a certain structure is visible whatever the method of fixation or treatment may be. A further consideration will be made in Section III, but we may draw here a tentative conclusion that the chromonemata exist throughout the whole cycle, but that in the living state, they are not clearly visible in the maximum development stage of chromosomes, owing to the fact that in this stage the chromosome matrix is in a highly condensed state as are the chromonemata.

## II. Chromosomes in Meiosis

Many years ago, FARMER (1905, 1912) stated: "Thus the essential peculiarities of the meiotic phase can be explained as follows: they are due to the coherence in pairs of premeiotic chromosomes and to the intercalation of a special form of chromosome-distribution during the course of what would not differ materially from an ordinary premeiotic mitosis". KUWADA (1937 a) re-examined this view on the morphological nature of the meiotic division under the new light of recent investigation and has reached the same conclusion. From this view of meiosis, it would be expected that the "spiral stage" exists also in the meiotic division, but until recently it had not been emphasized in botanical literature (NEBEL and RUTTLE, 1936). KUWADA and NAKAMURA (1934) have pointed out that "it seems probable that there are cases in which it has been over-looked or misinterpreted", and have mentioned that SINKE (1934) has observed this stage in the heterotype prophase in *Sagittaria* and KATO (1935) in the homotype division in *Tradescantia*. That the early meiotic structure and the early second prophasic structure are spirals has also been reported by NEWTON (1927) and TAYLOR (1931). More recently NEBEL and RUTTLE (1936) have observed the meiotic "spiral prophase" in *Tradescantia* and *Trillium*, and HIRAOKA (p.l.) more closely tracing the stages that precede and follow, in *Trillium*, *Tradescantia*, *Vicia* and *Psilotum*. In animal cytology this stage has been known as the prochromosome stage (KUWADA and NAKAMURA, 1934 b; KUWADA, SINKE and NAKAZAWA, 1939). Further investigations will doubtless discover this spiral stage in meiosis in many plants.

In *Trillium*, according to HIRAOKA, the nucleus before the spiral stage is full of the fine spiralized chromonemata of a definite polarized orientation, probably as a continuation from the preceding telophase. When the spiral stage is reached, each chromosome of the diffused state becomes contracted, so that the nucleus itself is rendered apparently less dense and appears clearer (Fig. 7). The polarized orientation has dis-

appeared in this stage, however, and the general straight arrangement has become lost. In the condensed chromosomes the spiral is visible more or less clearly. A stage follows then, where the spirals are drawn out—the stage which is known as the “unravelling stage” in animal cytology. When the unravelling takes place to its maximum extent, the leptotene stage is reached. The threads are now thicker than before the spiral stage. They appear not to be homogeneous in structure. The granular appearance is interpreted by KOSHY (1934) as due to each leptotene thread being “composed of two chromonemata twisted on each other”, and by NAITHANI (1937 *b*) as “the optical images of the spiral twists of the threads which have been exaggerated by the action of the fixatives”. NEBEL and his co-workers have observed a quadripartite nature of the leptotene thread, and others a bipartite nature (KAUFMANN, 1931; KOSHY, 1934), but most of the authors coincide in the view of

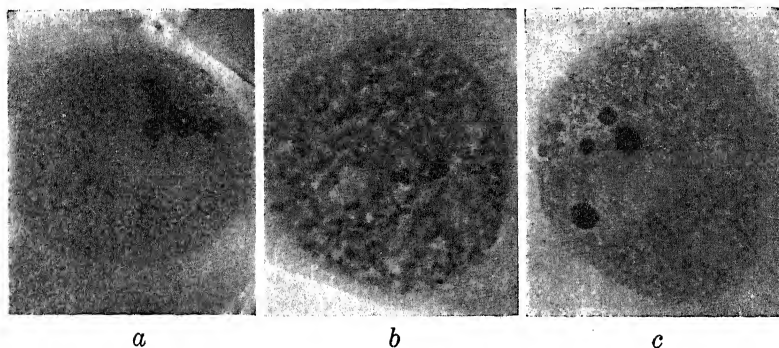


Fig. 7. Pollen mother cells of *Trillium* from acetocarmine smear preparations. *b*. Contraction stage (“spiral stage”). *a* and *c*, showing the stages before and after contraction stage respectively. (After HIRAOKA).

that the leptotene threads are single, at least in their appearance. In the pachytene, the two homologous elements are found closely associated along their length. The granular appearance of the thread is more conspicuous in the pachytene than in the leptotene. According to NAITHANI (1937 *b*), in well-fixed preparations the threads show a spiral structure in which they are coiled into a spiral of short pitch. SINKE (1934) has also observed the spiral structure in the pachytene. DARLINGTON (1935) and SAX and SAX (1935) are of the opinion that the chromosome splitting and the new spiralization occurs in the late or at the end of pachytene. In the diplotene, where the chiasma formation and the double nature of each element in syndesis becomes observable, the internal structure of the chromosomes is obscured (SINKE, 1934; NAITHANI, 1937 *b*). This obscurity may have some significance. In plants, the double nature of the elements in syndesis is lost to view again before reaching the diakinesis (HUSKINS and SMITH, 1935; comp. KUWADA and NAKAMURA, 1938), while in animal the tetrad nature of the bivalents is still clear in diakinesis.

SMITH (1932) has not expressly stated, but his figures seem to show, that the prophasic spirals with long pitch grow in diameter gradually up to the late diakinesis or metaphase where they attain their maximum diameter and are found considerably shortened. Similar spirals with long pitch have been illustrated by NAITHANI (1937 *b*) in his Fig. 7 showing diplotene stage, and described without any special remark, but it seems quite likely that these spirals are of different origin from those which he has observed in the pachytene and which he described as spirals of short pitch. The spirals in diakinesis and metaphase are much larger than those in somatic mitosis. HUSKINS and SMITH (1935) have called these spirals the major spirals. According to SMITH (1932), in *Galtonia*, two major spirals are contained in the chromosome, which separate from each other in the anaphase. The two spirals appear to form a double-stranded spiral, and if so, they should not be able to separate completely from each other "without a straightening out of their coils", but they do with their coils apparently intact. KUWADA and NAKAMURA (1933) observed some intermediate stages of separation in *Tradescantia reflexa*, and came to the conclusion that the two spirals are really not a double-stranded spiral, but a compound spiral so that the separation is possible just by their slipping out from each other. KUWADA and SINKE (KUWADA, 1938) have confirmed, on the other hand, in some few chromosomes of *Tradescantia reflexa* that the spirals are definitely relational spirals. It seems highly probable that both types of the double spiral, the compound and the double stranded or relational, may exist in *Tradescantia*, and that in the former case the component spirals are separated in the anaphase as is often observed, but in the latter the separation is postponed until later stages, when in *Tradescantia* the chromosomes are drawn out from their major spirals with subsequent thickening and shortening (KUWADA, 1938). MATSUURA (1938) has observed the inseparable condition of the double spirals in *Trillium* in which, since no telophasic unravelling takes place, there is no opportunity of separation before the second division; and thus he is led to the conclusion that a mechanism of crossing-over may be sought in this inseparable condition of the sister chromatids. At present, nothing more is to be said than what SMITH says: "It is difficult to conceive just how the separation occurs in the large chromosomes whose chromonemata make from two to three complete turns". Perhaps both compound and double-stranded spirals may occur in one and the same plant, but the frequency of each may differ in different plants.

In 1926 when KAUFMANN's paper drew keen attention to investigations on the spiral structure which had been so long neglected, FUJII made an important announcement as to the structure of the thread which form the meiotic large spiral. This is the discovery of the minor spiral, and by this discovery many important peculiarities of meiotic chromosomes have become adequately explainable. The original paper is written in Japanese and is known to European and American authors only through the citation by some Japanese authors. It seems advisable

here briefly to review the part of the paper concerning the spiral structure of the chromosome.

The paper concerns many important topics as indicated by its title: "Recent advances in cytology and its method of investigation". Under one of the headings, "the structure of the chromosomes", the author reports the results of his own observation with a historical sketch. Both staminate hairs and pollen mother cells of *Tradescantia reflexa* were observed, but chiefly the latter. In fresh material mounted with a cane sugar solution (6%) no chromosomes are observed in the division stage of meiosis. In the dark field illumination the region of the cell where the chromosomes should exist is quite dark or optically empty. FUJII has called this region the "dark pocket". When a drop of iron acetocarmine or a fixing mixture is added, the chromosomes become visible in the region of the dark pocket. This change is irreversible. When, however, a 0.06% acetic acid ( $\text{pH} = 3.47$ ) is used instead of the fixing mixture or acetocarmine, the same change takes place and is reversible. In some rare cases, fresh chromosomes are visible in the bright field illumination, and in this case the peripheral part of the chromosome is of higher density and more highly refractive than the inner part.

In iron-acetocarmine preparations, the chromosomes show the spiral structure from a stage considerably earlier than diakinesis to the anaphase of the second division. The spaces between the spiral turns are filled with a ground substance, and the whole body of the spiral which is far greater in density than the ground substance is found occupying the peripheral part of the solid cylinder of the ground substance by which the shape of the chromosome is determined. This structure of the chromosomes explains, the author states, the reason why the chromosomes may appear denser in the peripheral part than in the inner in the fresh state. In the very early stage of the meiotic prophase and in the stage which is rather close to the resting stage, the nucleus is also found filled with fine spirals.

The chromomeres which SANDS (1923) has observed and drawn as such are regarded as optical sections of the spiral. In order to show this, three optical sections of a chromosome in side view have been shown (Fig. 8 c). In the middle section the chromosome appears to be of the chromomeric structure as drawn by SANDS, but in the upper and the lower section it presents a continuous spiral aspect in which the direction of the spiral turns is opposite in the two sections, as would be expected on the assumption that its structure is a spiral. After a closer observation of the structure (Fig. 8 a) and a comparison of the spirals with those in other stages in meiosis (Fig. 8 b), the author comes to the conclusion that the spiral in the meiotic chromosomes is not a simple spiral as thus far assumed, but a spiral in which the thread itself is also a spiral; in other words, in the heterotype division the spiral of small diameter or the "primary spiral" is coiled "secondarily" to form the spiral of a greater diameter. It has been also suggested in this connection that there might be a third spiral which should really be called the primary spiral—a spiral which probably, it seems to the present writer, cor-

responds to the "minimum spiral" assumed later by DARLINGTON (1935) as a spiral not directly visible. In this paper, many other important suggestions have been made. Some of them which more directly concern the problem of chromosome structure may be mentioned below.

From the chromonema theory of the chromosome structure, the chromosome constrictions as well as the satellite may be explained as due to the fact that in these regions of the chromosome the coils are drawn-out or straightened.

It is easily possible to take the spiral turns erroneously for the chromomeres.

The shortening of the chromonema by coiling in the metaphase and the loosening of the spiral in the resting nucleus are regarded as reversible processes taking place between the two stages in one of which the dispersity is in a certain sense minimum and in another it is maximum. This idea of mitosis as a series of reversible processes, gelation  $\rightleftharpoons$  peptisation, was put forward first by FUJII (1921) and later by SCHAEDE in a more general form (1926, 1928). KIESEL (1930) says: "Die Vorstellung über die Gelbildung beim Entstehen der Chromosomen ist sehr verbreitet und hat sehr viele Anhänger".

The chromosome ground substance which FUJII has termed later the "hyalonema" has been regarded as a protective colloid by which each chromonema is protected from direct contact with others that may give rise to the "crossing-over" and other aberrations. In the limiting membrane of the chromosome, he adds, lipid may also exist, so that the chromosomes may come in contact with each other, and yet not fuse

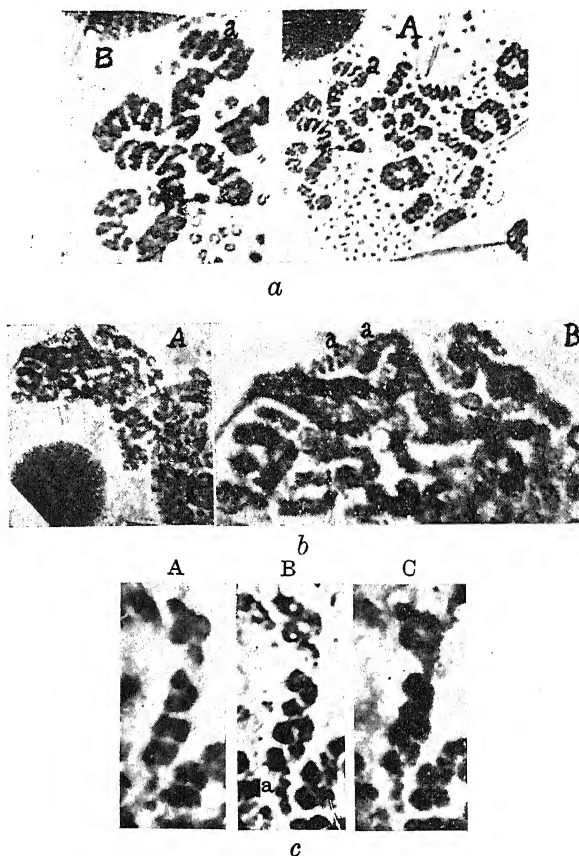


Fig. 8. Chromosomes in *Tradescantia* pollen mother cells. *a*. Diakinesis: B, an enlargement of the left hand side of A showing minor spirals at *a*. *b*. An interkinesis: B, an enlargement of a part of A on upper left showing spiral threads at *a*. *c*. Three optical sections of a part of a chromosome in prophase I, pushed out of the cell. A, surface view; B, middle optical section; C, bottom view. (After FUJII).

together.

In this paper, the author takes every opportunity of emphasizing that further investigations are needed before the chromonema is taken for the natural structure, but points out that the theory explains the individuality of the chromosomes more adequately than the other theories do. In conclusion he states that the chromosome multiplies by its own splitting as a cell is brought forth from a cell and a nucleus from a nucleus—"Omne chromosoma e chromosomate".

The double-coiled structure of the meiotic chromosomes was later confirmed and briefly reported by ISHII (1931) with drawings which are rather diagrammatic, and more fully by KUWADA and NAKAMURA (1933), DARLINGTON (1935 *a*) and others. In the case of small chromosomes, the major spirals have been observed by OURA (KUWADA, SINKE and OURA, 1938) by means of artificial chromosome unravelling, but the minor spirals have not yet been clearly demonstrated.

While this structure has since been observed in the cases of large chromosomes by many other investigators, the existence or the form of the minor spiral is questioned by some authors (HUSKINS, 1937). It is indeed difficult to determine owing to its minuteness whether the structure is a regular spiral, or a wavy or zig-zag corrugation, but it seems certain that some such structure exists. This structure becomes observable more clearly when the major spiral is drawn out. The coiling of the minor spiral is more regular when the uncoiling of the major spiral takes place in the natural state (Fig. 12 of KATO and IWATA, 1935) than when it is made artificially (Figs. 1 and 2 of KUWADA, 1938). In the former case the general appearance of these chromosomes drawn out from their major spirals is quite similar to that of the chromosomes in the second division (*Tradescantia*) or somatic mitosis. It may perhaps be assumed that in the double-coiled state the minor spiral may not be a regular spiral, being prevented from the becoming regular by the stress due to the existence of the major spiral, but becoming regular, at the same time increasing in diameter and correspondingly decreasing in length, when it is released from the stress by the uncoiling of the major spiral. It seems not to be of fundamental importance whether the minor spiral is a regular one or not (KUWADA, 1938).

It is highly probable, on the other hand, that the double-coiled structure is not of universal occurrence. When the chromosomes are considerably shorter in meiosis than in mitosis, it is highly probable that they are double-coiled ones, but when the two chromosomes are of about equal length (comp. MINOUCHI and KISHIMOTO, 1931), they should be of the same structure. They both may be of the double-coiled structure as in the case of *Galtonia* studied by SMITH, or both may be of the single-coiled structure.

In *Lilium*, the major spiral is often found uncoiled in the anaphase I, especially in the proximal part of the chromosomes (KATO and IWATA, 1935). KATO and IWATA have found a case where the major spirals are almost completely uncoiled in all the chromosomes of the anaphasic complements (Fig. 6). This suggests that there may be cases where the



major spiral has been uncoiled before the minor spiral is fully developed just as the old spirals in the somatic prophase are uncoiled as the new spiralization progresses. If this is actually the case, the meiotic chromosomes may be of the single-coiled structure. The double-coiled structure may not be a characteristic feature of meiosis. Whether the result is a double-coiled structure or a single-coiled one seems to depend on the nature of the matrix substance which controls the form change of the major spiral. This consideration forces us to the conclusion that the major spiral precedes in origin the formation of the minor spiral. KUWADA (1935) has discussed this question from another view point and has reached the same conclusion. In this view of the origin of the major spiral, it is an advantage, that the spiralization is assumable as occurring invariably in the chromonema, irrespective of whether the resultant spiral is single coiled or double coiled. SMITH (1932) has traced the development of the major spiral in *Galtonia* from the middle prophase to metaphase. The pachytene threads seem to be transformed gradually into spirals. It seems highly probable that here pre-leptotene spirals reappear in the later prophase by the contraction of the chromosomes after passing through the leptotene and pachytene stages where they are drawn out without a strong untwisting. We are led to this inference when we compare this case of SMITH in meiosis with that of the same author in mitosis where the spirals corresponding to those of the spiral stage (or the old spirals of the preceding telophase) in other plants reappear in the anaphase after they have passed the prophase stage where they had been drawn out. The major spiral may thus be the premeiotic spiral in its origin. The meiosis is also a mitosis, and it seems natural that in the prophase I the new spiralization takes place as in ordinary mitosis. We must, then, conclude that the minor spiral must exist. The major spiral may be straightened out, and hence the minor spiral alone could exist, but the major spiral alone can not. If the major spiral is observed in metaphase, this spiral must be a double-coiled spiral. The fact that in mitosis too the chromosome spiral may be double-coiled in the anaphase (*Galtonia* of SMITH) suggests that whether a spiral is single-coiled or double-coiled is a question of the manner of contraction of the chromosome, and not a question of the type of division. In both mitosis and meiosis, both single- and double-coiled structures should occur according to the peculiarity of the matrix which determines the mode of the chromosome contraction. The double-coiled structure should not be peculiar only to meiosis.

HUSKINS and SMITH (1935) have observed the "tertiary split" in the chromosomes in pro-metaphase in *Trillium*, in which the authors have not observed the minor spirals. In the photomicrograph of the abnormal, single-coiled anaphasic chromosomes in *Lilium* of KATO and IWATA (1935), this split is also clearly visible (Fig. 6). In this latter case, the split chromosomes (half-chromatids), each of the spiral structure, are found twisted around each other as observed by AISIMA in the chromosomes in mitotic anaphase. The tertiary split corresponds to the anaphasic split in mitosis, and hence it is natural that both chromosomes

are of the same structure and present the same configuration (comp. Fig. 6 with Fig. 5). In *Tradescantia reflexa* this split is not visible in the I anaphase, but becomes visible, though fragmentally, when the major spiral is uncoiled artificially (KUWADA, 1938).

While the chromosomes shown in Figs. 5 and 6 present the same configuration, those observed by HUSKINS and SMITH are of a quite different configuration in appearance. They can be brought into harmony only by the assumption that in the latter the minor spirals are concealed.

After anaphase, the behaviour of the chromonemata is different in different plants. Three main types can be distinguished (KUWADA and NAKAMURA, 1933). In the first type (*Tradescantia*, KATO, 1935), the major spirals are drawn out to certain extent during the telophase so that the interkinesis nucleus presents a similar appearance to that of the nucleus in the interphase in mitosis. In the second type (*Lilium*, KATO and IWATA, 1935) the major spirals are again drawn out, but the minor spirals remain, keeping their regular form, and in the third type (*Sagittaria*, SINKE, 1934) both major and minor spirals undergo practically no remarkable change in their coiled state. There seem to exist other cases. All possible cases will thus be as follows:—

Metaphase I	Interkinesis	Metaphase II	Example
1. single coiled	single coiled	single coiled	May probably be found in animals
2. „ „	diffuse	„ „	<i>Trixalis nasuta</i> [MINOUCHI & KISHIMOTO] (?)
3. double „	double coiled	double „	<i>Sagittaria Aginashi</i> [SINKE]
4. „ „	single „	„ „	<i>Lythrum</i> [SINKE] (?)
5. „ „	„ „	single „	<i>Lilium</i> (with variations) [KATO & IWATA]
6. „ „	diffuse	double „	<i>Galtonia</i> [SMITH] (?)
7. „ „	„	single „	<i>Tradescantia</i> [KATO]

In certain animals (WILSON, 1925), and in the abnormal case of *Galtonia* (TUAN, 1931), cases of interkinesis are known in which no nuclear vesicle is formed. These are extreme cases where the intermediate stage between the first anaphase and the second metaphase is extremely abbreviated. In this case, no stage comparable to the prophase exists in the second division, since the katachromasis does not take place at all. In the case of *Tradescantia*, on the other hand, the katachromasis takes place to so full an extent that the nucleus at the katachromatic climax presents a similar appearance to the nucleus in somatic interphase, and correspondingly a prophase exists in the second division that is comparable with the mitotic prophase. According to KATO (1935), the major spirals become clearer again in the beginning of the second prophase—a stage which reminds us of the spiral stage. The spirals are drawn out as the threads contract and grow thicker, and by the further contraction the homotypic chromosomes which contain single-coiled spirals are formed.

In the metaphase and anaphase, the chromosomes are longitudinally bi-partite in *Tradescantia*, showing two single-coiled spirals in them (KUWADA and NAKAMURA, 1935). In *Galtonia* (SMITH, 1932) and *Gasteria* (TAYLOR, 1931) the behaviour of the chromonemata is the same in the beginning of the prophase as in *Tradescantia*, but the configuration assumed at metaphase and anaphase by the two elements produced by the tertiary split is different from the latter.

In *Galtonia*, SMITH describes the nucleus at the climax of katechromasis as of the structure of "a network of lightly staining fibers". It seems, therefore, highly probable that in this plant the interphase exists and the interkinesis belongs to the *Tradescantia* type. In this plant the behaviour of the telophasic chromosome unravelling is the same both in mitosis and meiosis, showing that in this plant the spirals observed by SMITH in the mitotic telophase are the major spirals. In his Fig. 46 showing an early II-prophase, large spirals which are comparable with the I-telophasic spirals in his Fig. 43 are visible. SMITH has not stated expressly, but his figures showing subsequent stages show that these spirals are drawn out to form the prophasic spiremes. The spiremes contract then, and become spirally coiled. "The chromosomes at this stage resemble very closely the anaphase chromosomes of the heterotypic division". In these chromosomes the spiral turns illustrated are very few in number, and thus, both chromosomes, the heterotypic and homotypic, must be of the double-coiled structure. SMITH has observed that these spirals (the major spirals) are double—hence the tertiary split is observed. His drawings show that they must be double-stranded spirals. The products of the tertiary split are, therefore, a double-stranded spiral with a common matrix sheath. This configuration conforms with the result of HUSKINS and SMITH (1935) which they obtained in the prometaphase of the first division in *Trillium*.

In *Gasteria*, according to TAYLOR (1931) the katechromatic process in the interkinesis proceeds to variable extents, but in the extreme case, "the whole nucleus loses in stain holding power, and the chromatic continuity of the spireme becomes interrupted". Thus the interkinesis in *Gasteria* is regarded as being of the *Tradescantia* type. The telophasic major spirals in the first division which reappear in the early second prophase are double spirals indicating the occurrence of the tertiary split. As the spiral elements grow thicker, the spirals are gradually drawn out as in *Galtonia*. The thick elements or the spiremes that have been drawn out from the spiral are found containing "marginal, or at least double peripheral chromonemata" between which twistings exist as illustrated in his drawings (his Fig. 5, b, Pl. XXIX). It is highly probable that these twistings originate in the construction of the double spirals appearing in the beginning of the prophase II, and thus, it may be concluded that the double spiral in this early prophase is the double-stranded spiral. In metaphase and anaphase when the chromosomes are contracted to the maximum extent they show each a relational spiral within. The forming of the spiral is connected with the contraction of the chromosomes, and it seems likely that in this case the contraction of

a certain mode has transformed the twisted chromonemata in the prophase into the relational spirals. Thus we see that the general method of formation of the second metaphasic spirals is the same in both *Galtonia* and *Gasteria*, but in the latter the spirals formed very much resemble those of *Tradescantia* of single-coiled structure while in the former they are double coiled. This difference between *Galtonia* and *Gasteria* may probably be due to the difference in the mode of the contraction of the matrix substance in the two plants. In *Tradescantia*, the prophasic twisting has disappeared in the metaphase and anaphase, and the two elements (half chromatids) are observed to be each a single-coiled spiral (KUWADA and NAKAMURA, 1935). It is highly probable that in the case of *Gasteria* and *Galtonia* too, the two elements are of spiral structure. Thus in *Galtonia* as well as in *Gasteria* the chromosomes in the second metaphase and anaphase should be of the double-coiled structure. It is a general phenomenon that in fixed material the minor spiral is not visible. It must, however, be pointed out that in *Gasteria* the chromosomes are of the somatic form, and therefore it is natural that the minor spirals should here be not well formed. The chromosome development in the second prophase may then be postulated as follows:—

1) In the case where the interkinesis is of *Tradescantia* type, the spirals (the major spirals) in the preceding telophase reappear in the very beginning of the second prophase. The spirals are then drawn out accompanied by the thickening of the spiral elements. The contraction of the elements continues, and the metaphase chromosomes are formed.

2) The contraction may take place in each of the two elements (half-chromatids) independently, so that the relational coils between the elements tend to disappear—*Tradescantia*; or may take place not independently but in common, so that the old double-stranded spiral is reformed. According to the degree of contraction of these two kinds, the chromosomes formed may be as short and thick as in the first metaphase—*Galtonia*, or slender as in *Tradescantia*—*Gasteria*. In *Tradescantia* the half-chromatids are free from the stress due to the contraction taking place in common, and the minor spirals are well formed and greater in diameter than those of *Gasteria* chromosomes with similar appearance.

3) In the *Sagittaria* type there is no prophase in interkinesis, and in the *Lilium* type either kind of anachromasis analogous to the types of *Tradescantia* and *Galtonia* respectively may take place.

Now we come to consider the question as to whether or not the thickening of the elements in the second prophase is accompanied by the new spiralization of the chromonemata, but this will be considered in Section IV.

### III. Apparent Changes in Chromosome Structure as Related to Water-content

FUJII (1926) has pointed out that the change in pH is one of the important factors intimately connected with the reversible changes, the gelation and peptisation, which he regards as the two characteristic

features representing the cycle of mitosis. This statement of FUJII has been expressed by STRAUB (1938) in a more direct form, thus: "Ganz ohne Zweifel spielt die Wasseraufnahme und -abgabe für den Formwechsel des Chromonemas eine wesentliche Rolle". Experimentally, STROHMEYER (1935) has demonstrated that the structure of the nucleus can be rendered visible or not visible simply by taking out or putting in water, or speaking for convenience's sake, by dehydration or hydration, whatever the mechanism or state may be, by means of hypertonic and hypotonic solutions. Repeating the same experiments on a broader scale, SINKE (1937, 1939) has found moreover that these changes experimentally induced can be observed in certain plants taking place in the natural condition in the guard cell nuclei of stomata corresponding to the amount of the stomatal opening. In the maximum aperture of the stomata, the nuclei are obscure in structure and highly refractive as in the nuclei of the young petal epidermis treated with a hypertonic solution, and they come to present the nuclear structure when treated with a hypotonic solution. In the minimum aperture, the nuclei appear to be hyaline and are less refractive, and come to show the nuclear structure when treated with a hypertonic solution. In the half-open state, on the other hand, nuclear structure is visible in the natural condition, which is identical with the structure that become visible when the nuclei in the maximum aperture of the stomata are treated with a hypotonic solution and those in the minimum aperture with a hypertonic solution. SINKE has also observed that in these plants the cytoplasmic current is active in the guard cells when the stomata is closed and stops when it is open, and that in *Vicia*, corresponding to these nuclear changes a change in amount of starch grains takes place in the guard cell, which causes the change in tonicity of the cell.

SINKE (1937, 1939) has thus distinguished four types of the resting nucleus: 1) Homogeneous nucleus I—a hydrated nucleus. The nucleus of this type becomes a dehydrated nucleus appearing homogeneous, the homogeneous nucleus III, when treated with a hypertonic solution, without passing any stage where the nucleus shows the chromonema structure. 2) Homogeneous nucleus II—another hydrated nucleus. This nucleus becomes the homogeneous nucleus III when dehydrated, passing through the stage where it is heterogeneous. 3) Heterogeneous nucleus. This nucleus shows the chromonema structure. In this type two subtypes exist. a) Found in plant cells. When dehydrated, the nucleus becomes the homogeneous nucleus III, but can not be brought into the hydrated state, the water supply into the cell being prevented by the cell wall pressure. b) Found in animal cells. The nucleus can both be dehydrated and hydrated. 4) Homogeneous nucleus III—the dehydrated nucleus. It shrinks and is highly refractive, the nuclear structure being hardly visible. When dehydrated, no change is perceptible, but when hydrated, it comes to show its chromonema structure, and finally is swollen and hyaline. The nucleus of this type is found only rarely in the natural condition. The nucleus of the guard cell of the stomata of the maximum aperture, mentioned above, is of this type.

The apparent structural changes in the guard cell nuclei in the physiological condition show that the conclusion which can be drawn from the experimental results obtained by STROHMEYER and SINKE, that the nuclear homogeneity is only an apparent change that has nothing to do with the disappearance of the structure, is reasonable. The nucleus may appear homogeneous or heterogeneous according to the physiological condition, but the homogeneity is an apparent one due to the change in water relation or the inter-relation of refractive indices of the elements of the heterogeneous structure. The heterogeneous structure which is visible in the guard cell nuclei is quite identical with that of the nuclei of staminate hairs of *Tradescantia* and of other materials where the chromonema structure has been confirmed by many authors not only by direct observation of the nuclei themselves but also by tracing their transformation from chromosomes at metaphase in both living and fixed materials. It follows, then, that the chromonemata must exist in the resting stage irrespective of whether they are visible or not. The disappearance is due merely to the change in water-relation between the chromonemata and the surrounding substance. In metaphase also, where the chromosomes are in the highly dehydrated state, the chromonemata are usually not visible in the living state, but SHIMAKURA (1934) has observed them in the pollen mother cells of *Trillium* in which the I-division is in progress in an artificial medium (sugar solution). In this case the chromonemata must have become visible owing to the condition, perhaps, that an artificial medium can never be isotonic in the strict sense and that in pollen mother cells the cell membrane is more elastic than in staminate hair cells, so that the former are capable of taking more water in than the latter. The fact that the karyokinesis progresses normally must show that the cell is living. The chromonema structure must be the natural structure. The disappearance is merely an optical phenomenon as in the resting stage, in no sense involving the material disappearance of the chromonemata. The chromonemata must exist throughout the chromosome cycle, though certain chemical or physico-chemical changes may take place in the interphase or the resting stage as indicated by their change in stain holding capacity.

Some authors such as SCHAEDE and STRUGGER have regarded the resting nucleus as a sol, but FUJII (1931) has emphasized the point that the essential structure must be maintained in view of the fact that genetics strongly requires the fact of chromosome individuality. In his later investigation with his collaborator, FUJII has reached the conclusion that the sol theory of the nuclear structure in the resting stage can no longer be held (FUJII and YASUI, 1935).

In metaphase and anaphase the gelation process is at the maximum, and in the telophase the peptisation process begins. The peptisation which is reversible takes place in the matrix part of the chromosome. In connection with this change in the telophase it is an interesting fact that, according to SCHMIDT (1936), in sea urchin (living material) the birefringence of the chromosomes disappears in the telophase. That the process of nuclear re-construction is connected with hydration is clear from the fact

that the metaphase chromosomes arrested from their polar migration by dehydration can never enter on the re-construction process, unless an artificial water supply is made (WADA, 1936; SIGENAGA, 1937).

In the interphase, or in the resting stage<sup>1)</sup> where the peptisation is generally speaking at its height, the condition of the chromosomes is by no means uniform in all the nuclei. In the staminate hairs of *Tradescantia reflexa*, NAKAZAWA (cf. KUWADA, SINKE and NAKAZAWA, 1939) has found that there are two kinds of nuclei, one of a fine structure and the other of a coarser structure (Fig. 9a, b). SINKE (l.c. and p.l) has found the fine structure in the resting nuclei in the cells of grown up marginal leaf hairs of *Tradescantia virginica*, and has demonstrated experimentally that the structure is rendered coarser by treating the hairs with a hypertonic sugar solution (Fig. 10 a, b). The change is reversible. The fine structure is restored when the material is treated with a hypotonic solution or water. He (1939) has also observed the same reversible change in nuclear structure by subjecting the material to a high temperature which produces dehydration, or by treating it with some other nuclear dehydrating medium

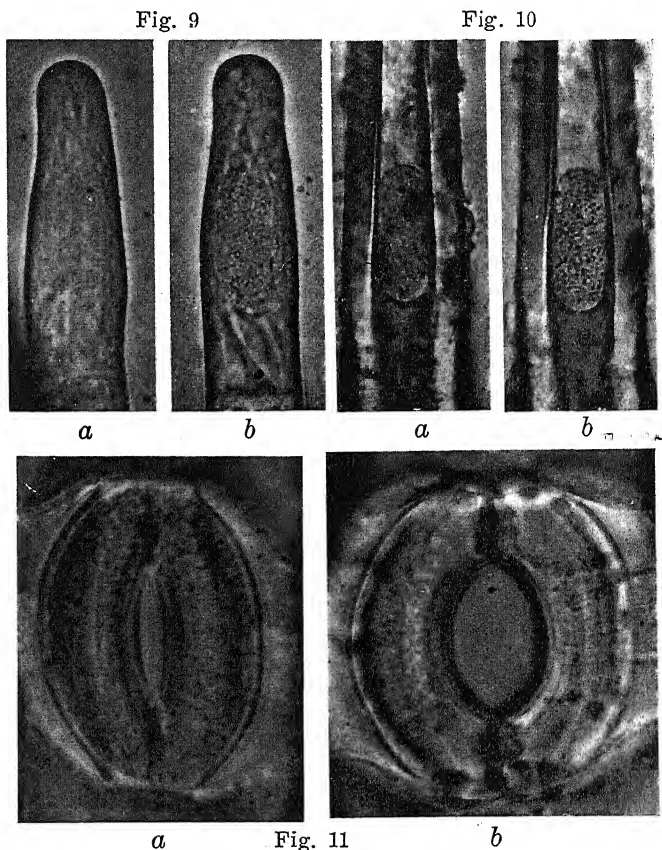


Fig. 9. Staminate hair cell nuclei of *Tradescantia reflexa* in living state (in liquid paraffin), showing fine structure (a) and coarse structure (b). (After NAKAZAWA, unpublished). Fig. 10. A marginal leaf hair cell nucleus of *Tradescantia virginica*. a, natural appearance of the nucleus in 0.1 M sucrose solution showing fine structure; b, the same when the medium is replaced with 0.8 M solution, showing coarse structure. Fig. 11. Guard cell nuclei in leaf epidermis of *Tradescantia virginica* showing fine and coarse structure. a, stoma in closed state; b, in open state.

1) The cases of the highly vegetative nuclei are out of the scope of the present review.

in the living condition of cells such as chloral hydrate, coal tar and ammonia (cf. SIGENAGA, 1937). SINKE (p.l.) has further confirmed that this transformation from the fine structure to the coarser or *vice versa* takes place naturally in the guard cell nuclei according to the change in magnitude of the aperture of the stomata, in proportion to which the change in tonicity of the guard cells should take place (Fig. 11 a, b).

From these results of experiments and observation it is conceivable that the two structures, fine and coarse, are due to the water content of the chromosomes. A further dehydration of the nucleus simplifies the structure further. This simplified structure closely resembles the structure of the nucleus at the late spiral stage (KUWADA, SINKE and NAKAZAWA, 1939). In the early spiral stage the spirals seem to be rather complex, suggesting that they are double spirals, and thus, the clearer figures of the spirals in the late spiral stage seem due to the union of the component spirals. If the simplification of the structure of the nucleus by artificial dehydration is due to the union of the spirals, the fine structure will be rendered coarser by the first union, and the coarser structure will come to resemble the structure at the late spiral stage by the second union. In the artificial dehydration, union may take place between chromosomes, but in a gradual, slow dehydration it seems highly probable that it takes place in the first place between intrachromosomal spirals lying in intimate spacial relation. If we assume that four chromonema spirals are contained in one chromosome, of which each two are sister spirals, the nucleus of the fine structure will be regarded as one in which all the four chromonemata are free from one another, and the nucleus of the coarser structure as one with two thick chromonemata produced by the union of the pairs of sister chromonemata. In the erythrocyte of *Triton* the nuclear threads and the anaphasic chromosomes are practically of the same thickness (SEIFRIZ, 1930, his Fig. 18). It is highly probable that in these nuclei all the chromonemata in the chromosome are united together. SINKE (p.l.) has demonstrated that this extremely coarse structure of the erythrocyte nucleus (*Triturus pyrrhogaster*) can be transformed by artificial means into a finer structure. We may call such a nucleus as that of *Triton* erythrocyte the spireme nucleus. The salivary nucleus of Diptera may be regarded as a special case of the spireme nucleus in which the chromonemata are more numerous. The facts that the spireme nucleus exists in the natural state and that the spiremes are as thick as the chromosomes in anaphase, may be taken as showing that the coarse structure is due to the union of the chromonemata in the chromosome.

Here we shall come back again to the telophase to look it on the assumption that the neighbouring chromonemata unite when the chromosomes are dehydrated. It is reported by many investigators that the telophasic chromosomes contain two chromatic strands. The two strands may twist around each other if some residuary "old" coils remain in existence, but may also lie almost parallel. The strands may show their spirality, but may also appear to be threads of a knotted structure. In the telophase these strands are found paired off, but in the interkinesis this paired distribution of the strands is rendered obscure, the general aspect



being of a diffuse distribution. The knotted appearance of the strands seems to represent the dehydrated form which the strands are caused to assume at metaphase and anaphase. Each strand probably represents two of the four chromonemata. When the nucleus is gradually hydrated in the telophase, the strands may come back to the spiral form—a hydrated form, and in the interphase single spiralized chromonemata may be visible. Such a nucleus will present the fine structure. In certain conditions the strands may remain without resolving themselves into single chromonemata through telophase and in interphase. The nucleus will present the coarse structure. In the latter case the strands may appear as knotted threads or spirals according to the degree or the mode of their contraction.

According to NAKAZAWA (KUWADA, SINKE and NAKAZAWA, 1939), the nucleus immediately before the spiral stage is of the coarse structure. The structure appears to be uniform throughout the nucleus, but when the spiral stage approaches, the uniform distribution becomes disturbed, suggesting that the chromosomes are here being contracted. If here also the coarse structure is due to the union of the chromonemata, and if the spirals of complex appearance in the early spiral stage are double spirals and are direct derivatives from the coarse structure as a result of mere contraction of the chromosomes, it must be assumed that the chromonemata contained in the chromosome are four in number, and the spiral of single appearance in the late spiral stage is in reality a quadruple spiral. If two chromonemata are assumed to be contained in the chromosome, the simplification in appearance of the spirals in the late spiral stage is not explicable, by the interpretation of the coarse structure of the nucleus as due to the union of the two neighbouring chromonemata. In the first meiotic division, it has been more clearly shown by HIRAOKA (p.l.) that the spiral stage is due to the contraction of chromosomes. KUWADA, SINKE and NAKAZAWA (1939) have expressed the view that the nuclear dehydration which causes the chromosome contraction in question is an outward expression of, or a secondary phenomenon due to, the factor that induces mitosis. The morphological term, the spiral stage, may be better replaced by "contraction stage" from the view point of mechanism, since in the preceding stage too, the chromonemata are in spiral. The difference between the two stages lies only in the fact that in the later stage the spirals are more easily recognizable than in the earlier stage, probably owing to the fact that in the former the chromosomes become contracted.

The fact that in the first meiotic division too, the chromosomes undergo a remarkable contraction in the beginning of prophase leads us to the conclusion that it is a natural consequence of the contraction that the leptotene threads appear to be single threads. In the experiments hydration causes the united threads to resolve themselves into the component threads, and thus the original appearance of the fine structure is restored, but in the prophase such a restoration does not take place until later stages. This depends probably on some change in the matrix substance that may be connected with the production of the substance of the new matrix. The physico-chemical properties of the matrix seem to differ in different cases, as suggested by the results of artificial unravelling. The

hydrated or dehydrated condition of the chromosomes would not be determined merely by the water content of the nucleus; it should be determined by the physico-chemical properties of the chromosomes themselves.

It seems now possible to harmonize the three opinions with regard to the number of the chromonemata in the chromosome. The divergency of the opinion seems to lie in the point that a clear distinction has not been made between the true and apparent numbers.

In meiosis, there is a conspicuous stage known as the "first contraction". This is in reality a second contraction. Two forms are known, the synizesis where no definite orientation of the chromosomes is shown, and the bouquet where a polarized orientation which reminds us of the telophasic orientation is presented. While in the first contraction ("spiral stage") each chromosome contracts, in the second contraction all the chromosomes in the nucleus together contract into a mass. It seems that this difference in the mode of chromosome contraction lies in the difference in colloidal condition of the chromosome matrix in the two cases.

In animals, after the diplotene, the chromosomes often show hydrated figures. This stage is known as the diffuse stage, and the typical diffuse stage has been observed in oogenesis. In the extreme type the hydration of the chromosomes seems to go so far that the granular structure of the chromonemata is visible. KOLTZOFF (1938) has distinguished these granules as "elementary chromomeres". SINKE (1937) has observed a similar structure in the salivary chromosomes artificially dehydrated. In plants, rather dehydrated forms are observed. The chromonema spirals which are observed in the pachytene are obscure in the diplotene. The double nature of the chromosomes in the diplotene becomes also obscure in the following stages, thus the tetrad nature of the bivalent chromosomes is lost to view. In metaphase and anaphase, the chromosomes are in a most dehydrated state in both plants and animals. In *Trillium* it has been observed that the complex reticulate structure of the chromosomes in diakinesis and pro-metaphase is rendered simply spiral in these stages (KUWADA and NAKAMURA, 1938).

In the case of the *Sagittaria* type of interkinesis, the chromosomes pass through the interkinesis without any remarkable form-change, but a hydrated form that is comparable with the form observed in the diakinesis is presented.

It has been observed that in the somatic prophase the change in concentration of the medium causes the nucleus to take the interphasic appearance (SIGENAGA, 1937). This case may be regarded as an abnormal cycle of mitosis in which the anachromasis and the katachromasis take place to a very limited extent. If this apprehension of the phenomenon is right, we may expect that in the following mitosis the chromosomes will appear twice as many in number as in the normal case—a phenomenon which may serve, if it should occur in nature (cf. WADA, 1932), as one of the mechanisms of WINGE's "indirect chromosome binding".

While the ordinary chromosomes undergo the reversible gelation-peptisation changes during mitosis, certain chromosomes or parts of

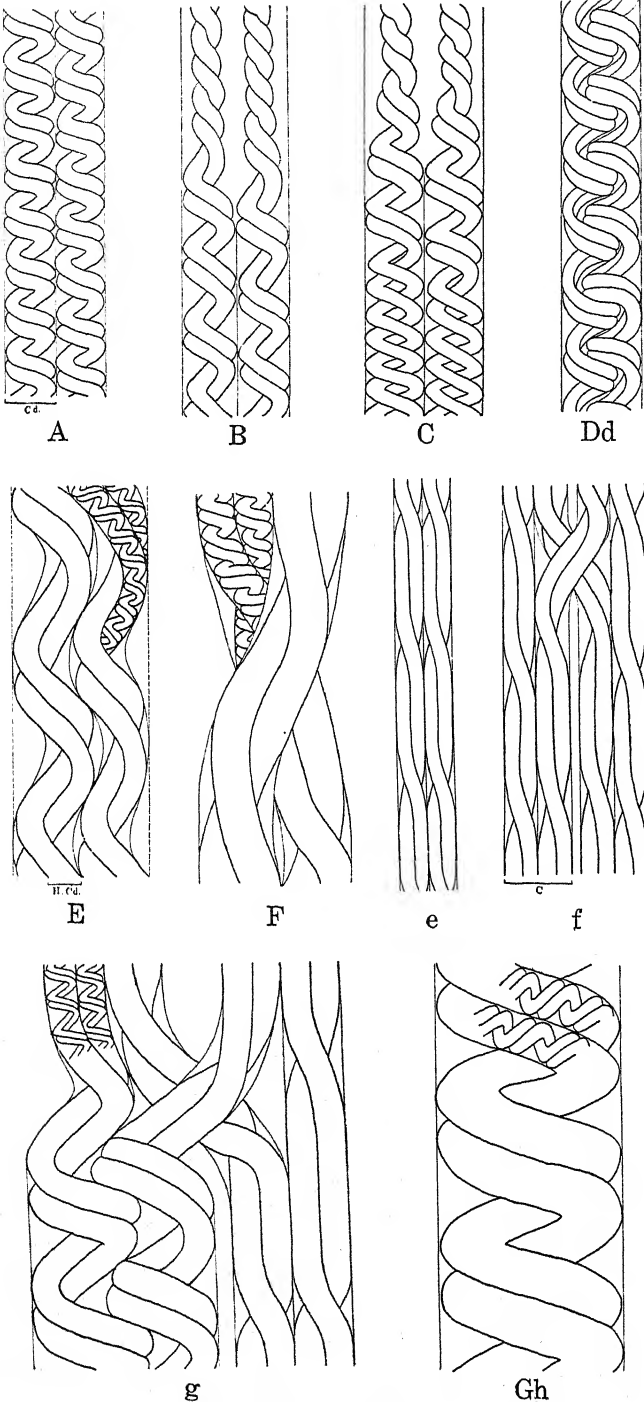
chromosomes do not, a phenomenon which is known as heteropycnosis. OURA (KUWADA, SINKE and OURA, 1938) has demonstrated that the heteropycnotic bodies can be unravelled by artificial means. The unravelled bodies show the spiral structure or a structure closely resembling that of the nucleus. From this fact it is concluded that the heteropycnosis is due to the peculiarity of the matrix substance which the chromosomes possess (KUWADA, 1937). STRAUB (1938) states: "Die besondere Matrix könnte verursachen, daß diese Chromosomenstücke ihre Spirale nicht oder erst später entwinden können."

A postulation may also be made about the ordinary chromosome matrix. We may assume that whatever the origin of the matrix substance may be (cf. ALEXANDER and BRIDGES, 1928), a new matrix is produced in each cycle of mitosis to form up the longitudinal halves of a chromonema into the individual chromonemata. FUJII (1933) has given the term, hyalonema to the matrix in the sense that the chromonema is imbedded in it and the whole structure is coiled into a spiral (cf. NEBEL, 1932*a*; GEITLER, 1935*b*). If, in the first cycle, the two daughter chromonemata each furnished with hyalonema are not separable to form the daughter nuclei, we may further assume that the hyalonema undergoes some chemical or physico-chemical changes in the following cycles to become different in contraction capability according to its age, and that it finally disappears. We call here the old hyalonema in which the spiral elements are imbedded the matrix. If the matrix belongs to the chromosome it may be called the chromosome matrix, and if it belongs to the chromatid, it may be referred to as the chromatid matrix, and so on. The chromatid matrix may contract in conditions in which the chromosome matrix can not. On this supposition the chromosome cycle will be explained in the next Section.

#### IV. Chromosome Cycle

At the present state of our knowledge it is a question whether the chromonema spiral belongs to the orthospiral type or the anorthospiral. DARLINGTON (1935) assumes that the chromonema spiral is of the type of the anorthospiral. We shall assume for the present that it is the orthospiral with turning points and with the numbers of the spiral turns of opposite directions which may not be equal owing to some free torsion that may occur in the end parts of the chromonema (KUWADA, 1937*b*), and also we shall assume that the chromonemata contained in the chromosome are four in number; and we shall try to postulate a theory of the chromosome cycle below. We shall begin it with mitosis starting at anaphase.

*Mitosis* (Fig. 12, A—G). In the anaphase, the chromosome contains two chromatids which are imbedded in the chromosome matrix. Each chromatid contains a double stranded spiral of the sister chromonemata which are imbedded in the chromatid matrix. Each chromonema has its own matrix, the hyalonema. The two chromatids run parallel or are twisted around each other in some measure or coiled into a double stranded spiral. While in the former two cases the whole structure may be called a single-coiled structure, in the third case it is a double-coiled structure.



In the anaphase and the early telophase, the chromosomes are in a highly dehydrated state, and consequently, the inner structure is not clearly visible in the living state. As the telophase proceeds, the chromosome matrix undergoes a peptisation process, and some inner structure becomes for the first time visible. In the case of the double coiled structure, the major spirals tend to be drawn out, and the two chromatids are rendered more distinctly separate and more clearly visible than in the preceding stages. In the case of the single-coiled structure, the two chromatids lie almost parallel with each other. The spirality of the chromonemata may be visible, but may not if the spiral is in a drawn out state. In the latter case, the chromatid appears to be a knotted strand. Each knot perhaps represents here a somewhat shortened and thickened turn of the united chromo-

nema spirals drawn out. In this case the chromosome appears to consist of two parallel elements showing a clear space between them—the telophasic chromosome split. The chromatids in the anaphase chromosome may also present the same configuration as in the telophase showing a chromosome split between them (comp. Fig. 38 of MERRIMAN, 1904). When the spirality is visible, the corresponding turns of the two chromatids form a series of lozenge-shaped spaces between the chromatids, the opposite angle of the turns coming closely together (cf. Fig. 27 of KAUFMAN, 1926). When the peptisation of the chromosome matrix goes further and is accomplished in all the chromosomes and in all their parts, the interphase or the resting stage is commonly reached.

In the interphase and the resting stage, the nucleus presents different appearances according to the extent to which the different matrices undergo peptisation. If only the chromosome matrix undergoes this change, and the chromatid matrix does so only to a slight extent or even not at all, the chromatids appear to be of the knotted strands, somewhat irregularly winding, or some such structure. These nuclei are of a coarse structure. If, in this case, the chromatid matrix undergoes peptisation to a greater extent or a hydration, the chromonema spirals become visible. The sister spirals may be the approximated ones or the separated ones according to the degree of the matrix change. In the latter case the nucleus is of the typical fine structure. Intermediate structures exist, and even in one and the same nucleus the structure may be fine or coarse or intermediate

Fig. 12. Diagrams to show the plausible hypothesis of the structure of chromosomes at different stages in mitosis and meiosis. Unless otherwise indicated, only a part of one chromosome is shown. c., chromosome; cd., chromatid; h.cd., half-chromatid. In the diagrams the outlines of the chromosome, chromatids and half-chromatids are shown to be smooth, but may be wavy as seen in Fig. 1a of GEITLER (1935a), according to the contraction state of the matrices. A-G, mitosis; d-h, meiosis. A. Anaphase. The spirals (and also the minor spirals in Gh) may assume the "knotted" configuration shown in the upper part in B (comp. Fig. 38 of MERRIMAN, 1904). B. Telophase (comp. Fig. 27 of KAUFMANN, 1926). C. Interphase. Upper two thirds showing coarse structures of different configurations and the remaining third part fine structure. Dd. Contraction stage ("spiral stage"). E. Prophase in later stage, showing the old spirals of the contraction stage in the process of transformation into the twisted form by their drawing out. New spirals are shown in part in the half chromatids of one of the two chromatids. F. Ditto in still later stage. Intertwining between the chromatids as a result of uncoiling of the old spirals is shown. e. Leptotene, showing the threads drawn out from the old spirals shown in Dd. f. Two homologous chromosomes in early prophase forming a chiasma. Chromatids and half-chromatids are shown. According to whether twisting exists or not between "partners" (chromatids), various types of compensating and non-compensating chiasmata will result. g. Later stage. New coils are shown in part. In the lower part of a univalent showing how the major spirals are formed by their transformation from twisting (comp. Figs. 24-26 of SMITH, 1932). Gh. A chromatid of double coiled structure in anaphase I or an anaphasic chromosome in II-division and in mitosis of the same structure. In prophase II, the twisting between half chromatids in the drawn out state is transformed into the spiral form (major spiral), as shown in g or disappears as the half chromatids contract, according to the mode of contraction (see text). In case the chromosomes are of double coiled structure in mitosis, the old major spirals or twistings between chromatids disappear when the chromatids contract in the prophase after the drawing out of the spirals, and the old minor spirals are transformed into new major spirals the new coils becoming the minor spirals.

in different parts of the nucleus. Thus it is generally difficult to determine whether a nucleus belongs to the type of the coarse structure or that of the fine structure. In the case of the spireme nucleus both chromosome and chromatid matrices undergo no remarkable change.

In respect of the structure, the nucleus in the interphase and that of the resting stage are not clearly distinguishable unless the latter is highly vegetative. The structure of the nuclei of highly vegetative nature is out of the scope of this consideration.

The peptisation change of the matrices in the interphase and the resting stage is reversible. In the beginning of the prophase a dehydration of the nucleus takes place at least in certain cases, probably varying in degree in different cases, in connection with the activation of the factor which induces the mitosis. The nucleus in this stage is of a coarse structure. The chromatid matrix which undergoes gelation shrinks and contracts, and the two chromonemata contained are brought into union. They are in a state somewhat drawn out from their spiral, and the chromatid forms as a whole a strand which has a knotted appearance and which is more or less irregularly wound. The chromosome matrix contracts then also, so that the chromatids reassume the spiral form. This is the beginning of the "spiral stage." As the contraction of the chromosome matrix goes further the two chromatid spirals become pressed into each other, thus the spirals appear to be a single spiral—a compound spiral. The nuclear dehydration is followed by hydration, and the chromosomes become distinct, standing out clearly from each other. Meanwhile, the new spiralization takes place, so that the two chromonemata in the chromatid (each now split) become coiled. Each split chromonema may thus form a double-stranded spiral, behaving in spiralization as a single chromonema, or a double spiral of mixed type, the compound and double-stranded, a part or parts of each split half being subjected to independent spiralization. The new spirals of the chromonemata may, therefore, be of the genuine type of double-stranded spiral throughout the whole length, or may be of the mixed type. The extreme case where the spiral is throughout the compound type may also occur. As the daughter chromonemata, each furnished with the new hyalonema, grow, and their spirals are transformed into regular spirals of increased diameter, the old hyalonema becomes the continuous matrix for them—the half-chromatid matrix. The whole structure is the half-chromatid which is in turn imbedded in the chromatid matrix. While the half-chromatids grow thicker, the old spirals which are now the half-chromatid spirals are drawn out. The old spirals are orthospirals (double-stranded spirals), and therefore, the drawing out causes untwisting. The untwisting may be incomplete, the process taking place very gradually, and some number of twists may remain linking the half chromatids not untwisted. These relic twists are observed in the anaphase, if the contraction of the chromosome in the prophase is due to the contraction of the half-chromatid matrix, as mere twists between chromatids which are in this stage no longer to be described as half-chromatids. If in the prophase, on the other hand, the chromatid matrix also plays a rôle in the contraction, the twisting as a form of the spiral is transformed into the spiral. In this case, the ana-

phasic chromosomes are of the double coiled structure. In metaphase and anaphase the chromosomes are in a highly dehydrated state, and consequently the chromonema spirals formed in the early prophase are not clearly observable in the living state. In the case of the single coiled structure, the twisting between the chromatids (the half-chromatids of the prophase) is greatly reduced or disappears in the telophase.

If the anaphasic twists between the chromatids remain not untwisted in the next prophase, and if in the contraction taking place after the straightening out of the old spiral, the chromosome matrix also takes part, the two chromatids will form together a double stranded spiral, and each chromatid will not be able to develop into an independent daughter chromosome. These daughter chromosomes then cannot be separated at anaphase. The chromosomes of this type of structure have been observed in meiosis, but in mitosis such a contraction as that due to the contraction of the common matrix does not occur in any case whether the chromosomes are of the single coiled structure or of the double coiled. In the latter case the relic twists (anaphasic twists) may reappear in the early prophase as the major spirals, but they disappear quickly when the chromatids contract after the new spiralization. The daughter chromosomes become, therefore, separable.

In the untwisting of the half-chromatids in the prophase, the two chromatids containing them will become intertwined with each other if in these chromatids the half-chromatid spirals or the old spirals are of the same direction. If, on the other hand, the old spirals are of opposite directions, the intertwining will not be made between the chromatids. Now, if the spiralization of the chromonemata is due to the intensification of the slight torsion preexisting in the chromonema (KUWADA, 1937*b*), it may be assumed that the spiralizations or intensifications repeatedly taking place in successive mitoses may cause a change in direction of the preexisting torsion, especially in the regions on either side of turning points, but the change would be very slight in extent in any two successive mitoses. The two sister chromonemata will, thus, be coiled, roughly speaking, in the same direction. The straightening out of the old spirals will, therefore, cause intertwining between the chromatids. The amount of the intertwining which remains and does not disappear in the late prophase is different in different cases. The direction of the intertwining may be the same or reversed in both arms of the chromosomes (SAX, 1936), because the original spirals have turning points.

If the chromosome cycle be as postulated above, we shall see that the classical view of the chromosome splitting, viz: that it occurs in the prophase of the division in the anaphase of which the daughter chromosomes are separated, is right, though the chromonemata which the chromosomes contain are split in the division preceding that division by two cycles. Another old view that the chromosomes are split in the telophase is also right in the sense that the telophasic chromosomes contain two chromatids with their own matrices. The "split" in this sense may be visible in anaphase and even in metaphase as reported by some authors. OVERTON's view that the chromosome is of an alveolate structure becomes also significant, if it

is assumable that four spiralized chromonemata are contained in the chromosome (cf. MARSHAK, 1936). As already pointed out by FUJII (1926), it is also seen that the theories of chromomeric and cylindrical structure of the chromosome are based on the misinterpretation of the spiral structure deformed by incomplete fixation or made difficult of observation by the change in refractive index. The old views become thus explicable under the single assumption that the chromosome contains four chromonemata coiled into an orthospiral, and by this assumption the difficulty of the longitudinal splitting of chromosomes which once threatened to be the death-blow to the theory of the spiral structure has now been also removed.

To recapitulate briefly the chromosome cycle postulated above, we may express it as follows:—The chromosome cycle is interpreted as being caused by reversible “gelation $\Rightarrow$ peptisation” changes. These changes are chiefly connected with the matrix substance of the chromosome. In the beginning of prophase the dehydration of the nucleus takes place followed by hydration which may not necessarily mean the hydration of the nuclear elements. In the metaphase the chromosomes are highly dehydrated, and the chromonemata contained are not clearly visible. In the resting stage the chromonemata are often subjected to hydration, and in this case the nucleus appears hyaline. The nucleus with visible chromonemata presents a fine structure or a coarse structure probably according to the hydrated or dehydrated condition of the matrix. In each cycle there take place the longitudinal splitting of the chromonemata, the separation of the daughter chromosomes, and the elongation (or drawing out of the spiral) and the contraction of the chromosomes which both seem to be intimately connected with the spiralization of the chromonemata. Probably a new matrix substance is formed and the old matrix (the chromosome matrix) disappears in each mitosis. The chromosome in metaphase may be long or short according to the mode of coiling, single or double.

The cycle with these changes is the normal cycle of mitosis. In the case of the heteropycnotic chromosomes the omission of the normal peptisation is the characteristic feature. In this case, it is not clear whether or not the new spiralization occurs in the prophase, but if the prophasic elongation takes place, it seems likely that it occurs. In *Sphaerocarpus Donnellii* in which the prophasic behaviour of the heteropycnotic X chromosome has been closely studied by LORBEER (1934), the X chromosome is elongated in the prophase, to judge from his drawings, to about three times its length in the resting stage, and moreover it tends to be stained less intensely in this stage than in the earlier stages, presenting a granulate appearance on its surface. If the spiralization takes place accompanied by the elongation of the chromosome, the following contraction of the half-chromatids will gradually reduce the twistings between them. The half-chromatids grow into the chromatids in the next cycle, a light line—the chromosome split—becoming visible between the chromatids in the prophase. They should now be separable from each other.

The fact that in the case of total heteropycnosis the chromosomes are in the condensed state in the early prophase as are the ordinary chromosomes in metaphase forces us to admit that the splitting can take place in



the condensed state of chromosomes as considered possible by NEBEL, but in the chromosome cycle postulated above, it is assumed preliminarily that the longitudinal splitting of the chromonema takes place in the beginning of prophase, as assumed by most authors.

*Meiosis* (Fig. 12, d—h). It seems more natural to assume that the behaviour and the condition of the chromonemata in the last premeiotic telophase and interphase are the same as in those stages of other mitoses, than to assume that there exist certain peculiarities in the last premeiotic stages. In the following hypothesis it is premeiotic chromosomes of such behaviour and such condition which are supposed to enter the meiotic cycle.

The behaviour of the four chromonemata in the beginning of the meiotic prophase is the same as in mitosis. The "spiral stage" occurs. After the spiral stage, the spirals are drawn out into the leptotene threads, their compound nature commonly not being perceptible. In this case the mechanism of drawing out of the spirals is not clear. In some cases the dual nature of the leptotene threads is observable (NEBEL and RUTTLE, 1936). The old spiral in the spiral stage is the orthospiral, and therefore, the drawn out thread is twisted. Untwisting should then occur. In the meiotic prophase every process being extremely gradual, syndesis may take place between the drawn out, homologous leptotene threads while they are in the process of untwisting. If the direction of twisting is the same in the two homologous threads, the untwisting will cause intertwining between the homologous threads in pairs (DARLINGTON, 1935*b*), and if the direction is opposite no such an intertwining will result. In the homologous chromosomes it is not to be expected that the direction of coiling should be the same in all cases, but if the spirals of the chromosomes are orthospirals, they should carry some number of turning points (KUWADA, 1937*b*), so that certain segments of one spiral of the pair are coiled in the same direction as certain segments of the other. If the untwisting of the leptotene threads takes place very gradually, and especially if it does not occur at the same time in each part of the whole length, but partially and successively segment after segment without any order or sequence of untwisting, there may be a chance or chances of untwisting simultaneously occurring in the segments of two homologous threads twisted in the same direction, and in this case intertwining will occur between the homologous threads as a result of the rotation of the segments untwisting. The intertwining causes the threads to cross each other at a number of points at which the "chiasmata" of DARLINGTON may be formed. Meanwhile the chromonemata are subjected to new spiralization. The spiralization may begin to take place before or after the formation of the chiasmata. If it takes place after the chiasma formation, a turning point or points should be formed between the chiasmata. The spirals thus formed are the minor spirals. They become visible first at the late pachytene or diplotene. The contraction of the chromosomes follows then to form the diakinesis chromosomes. This development of the diakinesis chromosomes is the same as that of the somatic chromosomes in mitosis. In the drawn out leptotene thread, some number of twists may remain not untwisted between the half-chromatids. If the following contraction of the chromosomes is due to the contraction of

the half-chromatid matrix, the contracted chromosomes will be of the single-coiled structure, the twists being reduced much in number and some being found to remain between the grown up half-chromatids (Fig. 6). If the contraction of the chromosomes is due to the contraction of the chromatid matrix, the contracted chromosomes will be of the double-coiled structure. In this case, the pair of twisted half-chromatids form a double-stranded major spiral without undergoing untwisting so intensely as in the former case during the chromosome contraction.

As to the reason why in meiosis the chromosomes are of double-coiled structure in the majority of cases, while it is a prominent feature of mitosis that they are of single-coiled structure, no adequate explanation is attempted at present, but it seems inferable that the contractile capability of the chromatid matrix being retained in the meiotic prophase may be connected with the fact that "the meiotic cycle is a much more leisurely process than the mitotic cycle." (SAX and SAX, 1935).

In the diakinesis, the two chromatids are commonly found with their major spirals pressed into each other, suggesting that in the chromosome contraction the chromosome matrix plays some rôle. If the anaphasic twists between the chromatids in the last premeiotic mitosis remain not untwisted in the meiotic prophase, the two major spirals of the chromatids will be interlaced with each other as considered above under "mitosis." In this case the major spirals are older in origin than those in the ordinary case where the two chromatids are freely separable from each other. If in this special case the prophasic untwisting does not occur at all, no intertwinning will necessarily be caused between the homologous threads, and in such cases the formation of the chiasmata in the sense of DARLINGTON may not be expected.

The major spiral is thus a modified form of the twists that remain not untwisted in the early prophase. It is a premeiotic spiral in origin. In the ordinary case it originates in the spiralization taking place in the last premeiotic prophase. The original number of spiral turns is considerably reduced in the untwisting in the meiotic prophase. A further reduction to the final number of turns of the major spiral may also take place as the minor spiral takes its full shape growing thicker in diameter during the chromosome contraction. The final number of turns is practically definite in any definite chromosome, being controlled by the contraction power of the chromatid matrix and the thickness and length of the half-chromatids which are definite in each definite chromosome. When the premeiotic spiral turns are reduced in number, the turning points in the spirals are also reduced, but some may remain in the region where some spiral turns remain in existence. It is a matter of chance how many turning points survive. The chances of turning points surviving in a greater number would be less than for a smaller number. It is also a matter of chance in which region of the premeiotic spiral the spiral turns survive. It would follow then that according to chance the direction of coiling of the major spiral may be the same or opposite in the sister chromatids (SINKE, 1934<sup>1)</sup>).

1) In this case, NEBEL's interpretation that it is a result of segmental interchange seems less probable (NEBEL, 1932b).

The two chromatid major spirals pressed into each other at diakinesis are separated from each other in anaphase except for the attachment point. The mechanism of the separation is unknown. The further development is different in different plants. In the case of the *Sagittaria* type of interkinesis, the chromosomes pass to the second metaphase without showing any apparent structural change except for a slight loosening of the spirals in the interkinesis, which is accompanied by an increase in diameter of the minor spirals and a corresponding decrease in number of the turns of the major spirals (SINKE, 1934). In this case, the whole stage up to the second metaphase is prophase in respect of the chromosome development. In the cases of the *Lilium* and *Tradescantia* types, on the other hand, katachromasis and anachromasis take place during the interkinesis, and in the extreme case (*Tradescantia* type), even the normal appearance of interphase is reached. In the *Tradescantia* type, the telophasic major spirals of the first division reappear in the second prophase. These spirals are drawn out and the drawn out threads are contracted as in the normal prophase. It is a question whether in this prophase the spiralization takes place or not. If in the contraction, the contraction of the half-chromatid matrix plays a principal rôle, the chromosomes of single-coiled structure are formed (*Tradescantia*), and if the contraction is due chiefly to the contraction of the chromatid matrix, chromosomes of the double-coiled structure result (*Galtonia*), and in the intermediate case the structure of *Gasteria* will perhaps be the result. In the case of *Lilium* type too, some of these cases may be expected to occur.

From the chromosome cycle postulated above, we see that the meiosis represents, though it is characterized by two successive divisions, merely one cycle of mitosis in the prophase of which the syndesis that accompanies the segregation division takes place as the characteristic feature. As in the normal cycle of mitosis, one longitudinal splitting, the growth of the split chromonemata and chromosomes, and the chromosome separation, take place during the course of the two divisions. The essential phenomena of mitosis are not hindered from taking place by the insertion of syndesis and segregation. It seems likely that any mitosis will be a meiosis, if the condition which gives rise to the pair of phenomena, the syndesis and the segregation, is fulfilled. Probably the "affinity" between homologous chromosomes, or the potential factor for syndesis exists in mitosis, but the syndesis is realized only when a realization factor as to the nature of which nothing is known at present acts on the potential factor (STRAUB, 1938; cf. SAX and SAX, 1935).

The insertion of the two phenomena, syndesis and segregation, in the prophase gives rise to a certain complication in the process of mitosis, not only that of the mechanical nature but also that of the physiological nature. The complication of the latter nature is probably most important for the further investigation aiming at revealing the nature of the realization factor. Many problems in connection with this question, are concealed in the prophase of the first division, but the development of interkinesis towards the "interphase" preceded by the stages of a katachromasis and followed by those of a corresponding anachromasis is also a problem in this

line of question. This problem is one left for further investigations as are the other problems, but in connection with the question of whether the spiralization may occur in the second prophase or not some consideration may be made on it from a mere morphological point of view.

If the interkinesis is considered merely in respect of the chromosome development, it may be regarded as representing the later part of prophase, but if it is considered from the view point of comparative morphology, it is regarded as a part of metaphase. In certain animals, it is known that the second metaphase directly follows the first anaphase without the formation of the nuclear vesicle (WILSON, 1925). This fact shows that in this case the segregation division takes place at the metaphase of the mitotic division; the interkinesis may exist in respect to time, but no interkinesis in the usual morphological sense. In many animals and plants, this interkinesis is prolonged in respect to time, and the prolongation is accompanied by the morphological changes analogous in variable degrees to the changes occurring between the two successive mitoses, the katachromasis and the anachromasis. In the extreme case these changes take place so extensively that there is found a stage in the transition which is comparable with the interphase of mitosis. Thus the two divisions, the meiotic segregation and the mitotic separation, tend to be each an independent division morphologically. If, in this case, the two divisions are transformed each into a perfect division, the chromonema and consequently the chromosome split should take place in each division. This is possible only when the syndesis does not take place and the segregation is no longer necessary, because the segregation and separation cannot take place both in one division. The divisions are no more meiosis but mere mitoses. In the normal meiosis, therefore, the chromonema split should not occur in each division, but the spiralization may occur. We have at present no evidence to show directly or indirectly whether it occurs or not in the second division. Any configuration of the chromosomes presented in the second metaphase can be interpreted as formed with, as well as without, the extra spiralization. The difference which could be considered between the two cases is only the difference in the origin of the spiral, new (homoeotypic) or old (heterotypic). In the case of *Sagittaria* type of interkinesis, it is, of course, highly probable that the spiralization does not take place in the second prophase. This case suggests that in the second division the spiralization is not a phenomenon that necessarily takes place. If the spiralization is a phenomenon connected with the growth of the divided chromonemata (HUSKINS and SMITH, 1935), it should not take place in the second division, as in this division the chromonemata are not split.

## V. Conclusion

Though our knowledge of the spiral structure of the chromosome is still meager, especially of the structure in the resting stage and in the heteropycnotic condition, and also in the knowledge of the chromosome matrix, we may perhaps say that no theory of the chromosome structure hitherto put forward could more adequately explain the chromo-

some individuality than the theory of the spiral structure. The chromonemata as components of the chromosome exist throughout the stages of the chromosome cycle. The configurations they assume appear very much different in metaphase and resting stage, but no fundamental change takes place in the spiral configuration they assume. The apparent difference is due mainly to the change in colloidal state of the matrix. That the nucleus is of the chromonema structure as well as the chromosomes has been demonstrated by KUWADA and NAKAMURA (1934) by converting metaphase chromosomes to the structure imitating the nuclear structure by treating the chromosome matrix artificially. Also no disintegration of the chromonemata such as that once assumed is caused by the chromosome splitting.

In certain stages the chromonemata may not be visible, but by this it is not implied that they have ceased to exist. They exist actually, but a change in water relation only makes them not clearly visible. They may appear to be a single strand, but may actually be a double strand or even a quadruple one. This is also an apparent phenomenon probably due to the change in water relation. The mechanism of this change is unknown at present, it may be more complex than mere experiment shows. More complex changes of chemical or physico-chemical nature cause the chromonemata to change in form stage after stage. This is obvious even from the fact that in the diffuse stages in which the interphase and the resting stage are also included, change in stain holding capacity takes place. But, the essential structure must remain unchanged throughout the cycle.

As was shown above, it appears that when it is assumed that four chromonemata are contained in a single chromosome, and that the spirals which they form are orthospirals, different opinions on many problems can be harmonized. If, on the other hand, an anorthospiral is here assumed, we encounter some difficulties, especially in explaining the mechanism by which the sister chromatids or homologous chromosomes are intertwined in the prophase. When an anorthospiral is drawn out, the thread is not twisted, and hence it does not rotate to cause the intertwining. When the spiral is divided into two daughter spirals along the longitudinal axis of the thread, the daughter spirals are freely separable, and no appearance of intertwining is expected to occur. Neither the drawing out of the anorthospiral nor the longitudinal division of the spiral thread can explain the intertwining figures which we observe (comp. Fig. 1).

The weak point attributed to the spiral theory, that is to say, the argument that the longitudinal splitting of the chromosome may cause the disintegration of the spiralized chromonemata can now be removed, though the longitudinal splitting of the chromonemata itself has not yet actually been observed. The theory would, then, stand on a firmer basis than ever. The spiral structure does, however, not represent the ultimate structure of the chromosomes. The recent advance in genetics still more decidedly requires the discontinuous structure of the chromosomes. The chromosome is of the spiral structure, but the spiral thread or the chromonema may be of the chromomeric structure (FUJII, 1931; FUJII and YASUI, 1935; HEITZ, 1935). The spiral theory is, therefore, expected to develop into a neo-

chromomere theory. It should perhaps, however, be emphasized here that the granular structure of the chromonemata which we actually observe may not represent this ultimate structure of the chromosome. The constancy in size of the granules at definite loci may be comparable with the constancy in thickness of the salivary chromosome bands at definite levels, where the thick bands have been demonstrated by ELLENHORN, PROKOFJEVA and MULLER (1935) by means of ultra violet rays to consist of thinner bands.

The further investigation from the view point of the neo-chromomere theory will perhaps afford the foundation of the real cytological explanation of the crossing-over. If the chiasma formation is determined by the segmental interchange of the homologous chromosomes as assumed by DARLINGTON, it seems highly probable that it is a question of the structure of the chromosome whether the chiasmata are of a random nature or regularly localized (cf. MAEDA, 1937). The mode of the distribution of the chiasmata should be determined by the distribution of certain structures in the chromosomes at which the segmental interchange can occur. If the leptotene thread is a compound thread consisting of two or four chromonemata, it will be required, in order that a regular crossing-over should take place, not only that the thread should not be spiral, but that it should be in a drawn out state at that stage as is required in any case, whichever the stage may be, at which the crossing-over takes place, and also that in the region where the segmental interchange takes place genes are absent. These regions may be of variable length in the chromonema, and the chromosome constrictions may be regarded as representing some of these regions that are of considerable lengths. The investigation of the chromosome constriction is thus important not only in connection with the problem of the origin of the nucleolus that may have a certain relation to the problem of the chromosome matrix, but also from the view point of the neo-chromomere theory of the chromosome structure.

If the mechanism of crossing-over is to be sought in the structural inequality of the chromosome body, SEILER's interpretation of the case of STURTEVANT's experiments with the ordinary and Nova Scotia *Drosophila* flies seems applicable without any special supposition such as "Kräftebeziehungen" between the factors. The classic interpretation of the crossing-over as "räumliche Beziehungen" may remain as the more reasonable one. The difficulty which SEILER (1922) encountered in his interpretation of the reason why Nova Scotia flies show the usual crossing-over will be removed, if it is assumed that in these flies an inversion has taken place in the second chromosome (TANAKA, 1934). There will come the time, it is also hoped, when the gelation-peptisation idea of chromosome form change can bring the neo-chromomere theory also to cover the chromosome structure in the primitive type of mitosis.

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## Recent Advances in Microtechnique II. The Paraffin Method in Plant Cytology

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### Contents

	Page
Introduction . . . . .	257
Fixation . . . . .	257
Washing . . . . .	261
Dehydration . . . . .	262
The paraffin solvent . . . . .	263
The transfer from solvent to paraffin . . . . .	267
Infiltration with paraffin . . . . .	267
Embedding . . . . .	268
The agar-paraffin method of embedding . . . . .	269
Preparation of the sections . . . . .	270
The staining and mounting of the sections . . . . .	272
Conclusion . . . . .	277
Literature cited . . . . .	278

### Introduction

At no time in the 20th century has the development of microtechnique advanced at such a rapid rate as during the last 10 years. Numerous refinements have been made in the old methods and several new methods have come to light. Although some of them are still in the experimental stage, it is worth while to take stock of the progress that has been made and make it more generally known than it seems to be.

The first article of the series (MAHESHWARI and WULFF 1937) dealt with a review of the technique for studying the development of the male gametophyte of Angiosperms. The present article is intended to call attention to the improvements and innovations that have been made during recent years in the paraffin method. Due to lack of space it has not been found possible to give complete schedules for each method; nevertheless, an attempt has been made to provide workable outlines of the more important ones, omitting such details which appear to be more or less generally known to workers in the field.

### Fixation

The first and the most important stage in the process is fixation. An ideal fixative will be one that (a) will penetrate rapidly and cause immediate death of the tissue immersed inside it, (b) will fix the cell

contents in such a way that they retain their original forms and positions, (c) will not unduly harden the material so that it becomes difficult to section after embedding in paraffin, (d) will provide a wide range of applicability by a regulation of the proportion of its ingredients, (e) will facilitate and not hinder the staining reactions that are intended to be used on the sections, and (f) will be reasonably cheap and convenient to use.

No substance is known which completely fulfills all the above requirements. We shall here call attention to a few which appear to be the best.<sup>1)</sup>

1. *Formalin-acetic-alcohol* (commercial formalin 5 cc, glacial acetic acid 5 cc, 50% alcohol 90 cc). This has a good penetrating power and is also an excellent preservative. The proportions of the ingredients can be altered to suit particular needs. The alcohol should be entirely omitted for most of the filamentous algae and fungi but its strength increased to 70% when dealing with aquatic angiosperms. In excursions this fluid is very convenient since material can be left in it indefinitely and excise authorities are not likely to take serious notice of it. It does not harden the tissues and the sections stain well with iron-haematoxylin, safranin and fast-green, and safranin and aniline-blue.

2. *Chromic acid*. Fluids containing chromic and acetic acids, dissolved in water,<sup>2)</sup> are more accurate in their action and materials fixed in such mixtures also give a brilliant stain with crystal violet, which is particularly useful for karyological studies on thick sections. Fleming's fluids, which contain some osmic acid in addition to chromic and acetic, remain unequalled even to this day in the faithfulness with which they fix the nuclei. For morphological and embryological studies, however, most workers avoid them because of their high cost, poor penetration and blackening effect, which necessitates the use of bleaching agents prior to staining.

3. *Bowin's fluid*. A favourite with the zoologists, the original formula has been slightly modified to suit plant material by the addition of a small quantity of chromic acid and urea.<sup>3)</sup> Four to six hours are enough for the fixation of small objects but a longer stay in the fixative does not do any harm.

4. *Corrosive sublimate-formalin-acetic-alcohol*. It has been strongly recommended by CHAMBERLAIN (1932) for material which offers difficulties in penetration and we have ourselves used it with satisfaction in several cases where due to a waxy or hairy coating the chromic acid mixtures failed entirely. For the formula and directions regarding its use, consult CHAMBERLAIN (1932).

5. *Carnoy's fluid*, made up with or without chloroform, is still used by several botanists on account of its rapidity of penetration and the

1) For formulae of most of the killing and fixing fluids, see CHAMBERLAIN (1932), McLUNG (1937) and LEE (1937); also ZIRKLE (1927), LEWITSKY (1934) & LA COUR (1937).

2) WATERMAN (1934) has suggested a method of carrying out chromic fixation in alcoholic media, but the results are not so satisfactory.

3) The addition of small quantities of urea or of some sugars, esp. maltose and lactose, is thought to improve the penetration of some fluids.

quickness with which the material can afterwards be brought into paraffin. There is no particular necessity for using absolute alcohol in the formula; 95% alcohol can be substituted for it without any disadvantage and many workers actually prefer to weaken it still further to about 80%. In the writer's opinion, this fluid is inferior to formalin-acetic-alcohol and the only advantage it offers it that it saves some time in dehydration.

6. *Chromo-formol*. The mixtures of formalin, chromic acid and acetic acid, which have come into use in recent years, are the best fixing fluids for general use. The presence of formalin causes rapid killing and its preservative action obviates the necessity of washing the material after 24 hours, as one has to do with the chrom-acetic mixtures. Material fixed and shipped to us in S. Navashin's fluid (1% chromic acid 10 cc, 16% formalin 4 cc, and acetic acid 1 cc) from Europe and America was still found to be in good condition for embryological studies. BRUUN (1932), who has made an extensive cytological study of *Primula*, reports that in some cases his root-tips had to remain in Navashin's fluid for two months before there was any opportunity of imbedding them. They did not, however, suffer in any way from such treatment.

The following formula, given out by the University of Stockholm, is now very widely used in India, America and other countries:—

Chromic anhydride . . . . .	1 gm.	} A
Glacial acetic acid . . . . .	10 cc.	
Water . . . . .	65 cc.	
Commercial formalin . . . . .	40 cc.	} B
Water . . . . .	35 cc.	

"A" and "B" are to be mixed in equal parts immediately before use. The mixture which is orange-yellow in colour, soon becomes darker and blackish-green. When this has happened, it is no longer usable.

RANDOLPH (1935) has recommended the following proportions for fixation of root-tips and smears of microspore mother cells:—

Chromic anhydride . . . . .	1 gm.	} A
Glacial acetic acid . . . . .	7 cc.	
Distilled water . . . . .	92 cc.	
Neutral formalin . . . . .	30 cc.	} B
Distilled water . . . . .	70 cc.	

As before, "A" and "B" are to be mixed in equal proportions just before use.

The value of the chromo-formol mixtures lies not only in the quality of their fixation but their wide applicability. LEWITSKY's (1931) detailed analysis of the fixing action of the components is of great value since it enables us to work more intelligently towards an alteration of these proportions, when this becomes desirable to suit particular requirements.

It must be emphasized, however, that even the best fixing fluid will fail to give satisfactory results if proper attention is not paid to other conditions that are requisite for good fixation. Some of these are mentioned below:—

1. The tissue or plant part that is fixed must be in a perfectly turgid condition at the instant of fixation. It is waste of time to fix post-mortem or pathological states.

2. As far as possible, fixation must be carried out in the field. When this is impossible, material should be brought to the laboratory in a pan of water. The dessicating effect of the electric fans in tropical laboratories and heaters in the West, demands that fixation must be completed as quickly as possible.

3. Plant in tropical countries are often in a state of partial wilting during mid-day and it is better to avoid fixation at this time. If it becomes necessary to do so, keep the twigs submerged in water for half an hour or so, prior to fixation. Plants, whose root-tips are to be fixed, should be watered well about 12 hours before fixation. For full directions regarding the preparation of root-tips for killing, see M. NAVASHIN (1934).

4. The material should be cut into small pieces in order to facilitate quick penetration. In the case of flower buds, the sepals and petals should be invariably removed and the anthers and ovaries, when large, should be cut up into smaller pieces. In the case of very small buds, which are in danger of being injured by such handling, the same object can be achieved by cutting down the tops to the desired level. Gymnosperm cones like those of *Pinus* should be dismembered. In the female flowers of *Ephedra*, not only the bracts but even the outer integument can be dissected away with ease (MAHESHWARI, 1935). For very critical work JEFFREY (1937) thinks that the pieces should not be more than 0.5 mm. thick.

Root-tips like those of onion and *Tradescantia* can be fixed entire, but the thicker ones should be sliced *under the fixative* into 2 or more pieces. Tips of some aerial roots have a mucilage on them which retards good fixation. This can be removed by soaking them for a few minutes in distilled water and then wiping the surface with cotton (BEARD, 1937), or by wetting them in the mouth with saliva and then drawing out gently between the lips. For his work on the microsporogenesis of sugarcane, SANTOS (1937) treated the flower buds in the latter way and got excellent results.

5. In the case of alcoholic fluids most objects sink readily (except *Azolla*, *Salvinia* and similar other plants which have considerable quantities of air in their tissues), but when dropped in aqueous fluids they show a tendency to remain floating on the surface and must be sunk by sucking out the air. Small hand pumps can be readily constructed (see JEFFREY, 1928, and DATTA, 1934) for use in excursions as well as the laboratory. A water suction pump is also quite satisfactory. Very powerful motor pumps do the work in a very short time but are likely to cause distortions.

TAHARA (1921)<sup>1)</sup> suggests that the materials (*Chrysanthemum*) should be first treated with Carnoy's fluid (having chloroform) for 15-30 minutes, then washed in 95% alcohol and dropped into the fixing fluid (Flemming).

1) Also in 1914 in a paper written in Japanese.

KIHARA (1924) recommends a shorter application of Carnoy's fluid for the fixation of cereals. This method has won great popularity but occasionally the results are conflicting. Thus, NEVINS (1933) complains that *Sphaerocarpus* thalli prepared in this manner showed an unusual degree of shrinkage. As pointed out by LACOUR (1937), material that has been pretreated with Carnoy's fluid should never be pumped, as this drives the alcohol and chloroform into the tissues and produces an inferior fixation before the chromic mixtures can penetrate. He recommends the addition of a small amount of saponin to all aqueous fluids since this reduces the surface tension.

In his work on the division of the generative cell and the development of the sperm cells of *Lilium*, the late S. G. NAVASHIN (1910) injected the fixing fluid into the styles. No one seems to have used this method recently but it deserves mention because of the reputation of this author and the excellent work he produced.

6. The amount of the fixing fluid should be about 50 times the volume of the objects. Plant tissues often contain so much water and other soluble substances that their outward diffusion may seriously alter the composition of the fixing fluid, if only small quantities are used. Fixing agents with alcohol (e.g. Carnoy's, formalin-acetic-alcohol etc.) will, however, fix a much larger proportion of material.

7. In most cases the fixation is to be done at room temperature, slight variations of heat or cold not making much difference. According to the directions given by most workers Allen's modification of Bouin's fluids (B. 15) should be used at a temp. of about 38°C. CHAMBERLAIN (1932) recommends hot corrosive sublimate-formalin-acetic-alcohol (80°C), LAND (1900) used boiling hot chrom-acetic acid and POPHAM (1938) got better results with hot formalin-acetic-alcohol than with Flemming's fluid. While such a procedure may occasionally prove advantageous, it is beset with the danger of producing artefacts and ought to be avoided as far as possible. With the chrom-formol mixtures there should be no heating; on the contrary the vials may better be kept in cool water in the summer season and protected from direct sunlight in the field.

### Washing

After the material has remained in the fixing fluid for about 24 hours it should be washed before further treatment. Older botanists uniformly recommended 24 hours in running water for all aqueous fixing fluids. LA COUR (1931) thinks that 6 hours are quite sufficient, specially if warm water be used. RANDOLPH (1935) says that after fixation in "Craf" (see formula on p. 259) the objects can be taken directly to 70% alcohol without any washing whatever. Similarly MCWHORTER & WEIER (1936) write that no elaborate washing is necessary and material may be transferred to dioxan (see p. 265) merely after rinsing out the excess fixative.

The writer's practice is to start the washing arrangement just before leaving the laboratory after the day's work, and begin the dehydration process next morning. This gives about 12 hours for washing which is

quite adequate for most objects. A twenty-four hour period seems unnecessary and may even be harmful for delicate objects.

With formalin-acetic-alcohol it is enough to make one change with 50% alcohol, another with 60% alcohol and a third with 70% alcohol, all at intervals of about 2 hours each. This washes and dehydrates at the same time. With corrosive sublimate-formalin-acetic alcohol the washing must be more thorough otherwise crystals of mercuric chloride are likely to appear in the sections. Tincture of iodine facilitates the washing. In the case of Bouin's fluid, material should be quickly run up to 70% alcohol and washed at this stage by frequent changes of alcohol till no more yellow colour comes out.

### Dehydration

The more delicate the material, the more gradual should be the process of dehydration. The writer uses the following strengths of ethyl alcohol for most objects:— 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 95% and absolute alcohol. It is important that the material does not remain too long in the weaker alcohols since this is sure to cause maceration. It is a good plan to start dehydration in the morning and bring the material to the 60 or 70% alcohol<sup>1)</sup> stage before leaving the laboratory in the evening. After this has been done it is not necessary to go to the laboratory at inconvenient times to change the fluids. One change in the morning and another in the evening will be all right for most objects.

Due to the high cost of absolute alcohol, many workers (specially in German and Austrian laboratories) dehydrate only up to the 96% alcohol stage<sup>2)</sup> and then pass over into mixtures of alcohol and benzol or xylol (for a full discussion of this question see KISSER, 1929). Indian laboratories can get absolute alcohol at a cheap rate (less than a rupee per pound) by first obtaining a permit from the excise authorities to buy it free of duty.

It is also possible to affect dehydration by means of glycerine (BEN HILL, 1916). The objects are put into 5 or 10% glycerine in a shallow dish covered with filter paper to keep out the dust. After a few days when the glycerine has become thick and syrupy, wash thoroughly in 95% alcohol and proceed as usual. The advantages of this method are that (1) dehydration is effected very gradually and uniformly without much attention on the part of the operator; (2) the material does not harden; and (3) if it be considered desirable to postpone the imbedding process, concentrated glycerine is a very satisfactory preserving medium for this purpose.

1) When one is on an excursion, it is inconvenient to take the whole series of bottles. Under such conditions it is best to use the "drop method" of adding 95% alcohol till the objects are in an alcohol of 70-80% strength. The remaining steps can be gone through after returning to the headquarters. As mentioned before, material fixed in Navashin's fluid can be allowed to remain in it for a week or two without any harm.

2) This is also advantageous in another respect, for it avoids the hardening due to the action of absolute alcohol. It is claimed by some workers that material dehydrated and embedded in this way is easier to section.



### The paraffin solvent

*Xylol*: Since alcohol does not mix with paraffin, it has to be replaced by some other substance which will act as a go-between. Xylol is most commonly employed for this purpose. Its boiling point is about 140°C and specific weight 0.88–0.89. At 20°C it dissolves about 12% paraffin of 58°C melting point.

The following series of mixtures of absolute alcohol and xylol will do for most objects: 10%, 25%, 50%, 75% and pure xylol. The 75% xylol should have a pinch of safranin or phloxine dissolved in it to colour the objects. This facilitates their orientation at the time of cutting.

The failure of many workers to get good sections of pieces of stems and roots of flowering plants, cones of Gymnosperms and similar hard objects is frequently due to the fact that they do not allow enough time for complete dehydration and infiltration and then try to compensate for this by keeping the objects for long periods (weeks and months!) in melted paraffin. It is true that a prolonged stay in xylol has some hardening effect on the tissues but long periods in the paraffin bath are far more disastrous. The writer never found it necessary to keep anything in the paraffin bath are far more disastrous. The writer never found it necessary to keep anything in the incubator for more than a week; in most cases 12–24 hours are quite sufficient.

*Benzol*: In many laboratories (particularly in Germany and Austria) benzol is used in preference to xylol. Its chief advantage is that it has a lower boiling point (80.4°C) and can consequently be got rid of more quickly than xylol. The disadvantage is that it dissolves a little less of paraffin (8% at 20°C) than xylol.

*Toluol*: This is used in exactly the same way as xylol and benzol but offers no special advantages and hardens the material more than other solvents (see RALPH, 1938, p. 14). The boiling point is 110°C.

*Chloroform*. In a number of laboratories chloroform is regularly preferred to xylol and benzol. The procedure is approximately the same. After the material is in absolute alcohol, pass it through the following mixtures: 25% chloroform, 50% chloroform and 75% chloroform followed by two changes of pure chloroform. The advantages of using chloroform are that it does not harden objects to the same extent as xylol, benzol or toluol, and its boiling point being only 61.2°C, it is very easily removed when the material is in the incubator. The disadvantages are that it is more expensive and has a poorer penetrating power so that it can be used with satisfactory results only on small objects like root-tips, flower-buds etc. The bottles and tubes containing chloroform or its mixtures should be kept in the dark, since it rapidly decomposes in strong light.

*Cedar oil*: Zoologists often make use of cedar oil and LEE (1937, p. 80) recommends it as the *very best* clearing agent for paraffin embedding. Its chief advantage lies in the fact that it does not cause any hardening. STEVENS (1930) experienced great difficulty with the "teleuto-

spore-horns" of *Gymnosporangium* which became flinty and refused to cut with the alcholo-xylol method, but good sections were finally obtained by the use of cedar oil. The chief objection against it is that it is almost impossible, to eliminate it from the paraffin in the final stages of embedding.

*Normal butyl alcohol*: This can dissolve small quantities of paraffin, and a mixture of equal parts of ethyl and butyl alcohols is also miscible with water. Working on this idea, ZIRKLE, in 1930 (see also LARBAUD, 1921, and MARTIN, 1922), proposed the following series for dehydrating plant materials:—

Solution No.	1	2	3	4	5	6	7	8	9	10	11
Water	95	89	82	70	50	30	15	5	0	0	0
Ethyl alcohol	5	11	18	30	40	50	50	40	25	0	0
Butyl alcohol	0	0	0	0	10	20	35	55	75	100	100
Percentage of alcohol	5	11	18	30	50	70	85	95	100	100	100

For material fixed in formalin-acetic-alcohol, the route can be shortened by washing it with 50% ethyl alcohol and then taking it directly to solution No. 5.

Recently LANG (1937) has suggested certain modifications in these proportions and recommends the following revised series of solutions:—

Solution No.	1	2	3	4	5	6	7	8	9	10	11
Water	95	89	82	70	57	43	30	18	9	3	0
Ethyl alcohol	5	11	16	23	28	30	30	27	21	12	0
Butyl alcohol	0	0	2	7	15	27	40	55	70	85	100
Percentage of alcohol	5	11	18	30	43	57	70	82	91	97	100

As suggested by Dr. Lang some intermediate steps should be interpolated in the first half of the series for more delicate material.

HEMENWAY (1930) has introduced the use of glycerine in the method and recommends the following schedule as one that is not likely to have any hardening effects on the material:—

1. Transfer fixed material directly or after treatment with HF to 10% glycerine and let it concentrate.
2. After the glycerine is thick and syrupy, drop the objects in a mixture of equal parts of glycerine and butyl alcohol.
3. Pure butyl alcohol.

The chief advantages of the Butyl alcohol<sup>1)</sup> method are that it works more quickly and does not cause much hardening.<sup>2)</sup> We think that

1) Butyl alcohol has another subsidiary use in that a mixture of approximately 10 parts of this and 90 parts of xylol is very effective in loosening cover glasses from old and discarded slides (CARLSON, 1935). More powerful still is trichloroethylene (OLTMAN, 1935).

2) The "Triarch Botanical Products" owned by Dr. G. H. Conant, regularly employ butyl alcohol as their paraffin solvent.

Lang's revised schedule is the best. To secure uniformly good results, the following precautions are necessary:— (1) Do not hurry through the series but allow the material to remain for at least 12 hours in each grade after the 50% alcohol stage has been reached; longer will do no harm. (2) Since butyl alcohol is not so readily miscible with paraffin as xylol, more time should be allowed for infiltration and the incubator should be well aerated so that the fumes of butyl alcohol are able to escape quite readily.

*Dioxan* (diethylene oxide): This is a colourless liquid with a faint odour, a boiling point of 100.8°C and a melting point of about 8°C. Its chief property, with which we are concerned here, is that it is miscible in all proportions with water and alcohol and can also dissolve paraffin, slightly when cold, but quite readily when heated.

Taking advantage of these properties it is possible to use it as a medium for taking over materials from water into paraffin. GRAUPNER and WEISBERGER (1931) of the University of Leipzig were probably the first to recommend its use but they tried it only on animal tissues. MCWHORTER & WEIER (1936), who have made the most extensive trials with dioxan on plant materials report that grading it like alcohol has no advantage. No elaborate washing out of the fixing fluid is necessary and the objects may simply be rinsed and dropped into dioxan.<sup>1)</sup> After two changes with fresh, pure dioxan (the time varies with different tissues but material left for several days does not harden), embedding may be done in paraffin in the usual way.

Since dioxan is only a weak solvent of paraffin, BAIRD (1936) recommends the addition of a few drops of xylol to it (5–10 cc. of xylol per 500 cc. of dioxan) immediately before the addition of paraffin. MOSSMAN (1937) criticises this step and thinks it to be illogical.

WHITAKER (1937) used dioxan on plant parts infected with aphids and got smooth ribbons. After the material was in pure dioxan, he passed it through the following mixtures: 4 parts dioxan + 1 part xylol; 3 parts dioxan + 2 parts xylol; 2 parts dioxan + 3 parts xylol; 1 part dioxan + 4 parts xylol; and finally pure xylol. In defence for this lengthy procedure he states that since xylol is a better solvent of paraffin, its use in the last stages insures more uniform penetration.

The following are claimed to be the advantages of dioxan:— (1) it eliminates washing after fixation, (2) it is time-saving and economical, and (3) it does not harden material like xylol and benzol. BAIRD (1936, p. 71), who has made much use of it on zoological material, writes: "While dioxan does not completely fulfill the dreams of a microscopist for a perfect dehydrating agent, yet it is definitely superior to any present known substance. Regardless of fixation, tissues retained a fairly soft consistency with a minimum of brittleness and distortion."

Nevertheless, its use is beset with serious difficulties:—

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1) There have been complaints of a shrinkage of tissues resulting from such a rapid change and it is therefore better to use three intergrades of water and dioxan (25%, 50% and 75% dioxan) before passing into pure dioxan.

(1) Dioxan vapour in the concentration of 1:1,000 parts of air is definitely poisonous. MAGRUDER (1938) warns that all work with dioxan should be done in a chemical hood because an inhalation of the fumes can give rise to severe bronchial, liver and kidney disturbances.

(2) It penetrates tissues slowly and dissolves paraffin only with difficulty. As a result, infiltration is often incomplete.

(3) It interferes with proper staining so that differentiation is difficult and the sections lack sharpness and brilliancy, the cytoplasm tending to remain overstained.

The evidence being so conflicting, one can only say that while there may be possibilities in this chemical, it has not yet passed the stage of experimentation. It is undoubtedly useful in animal histology as reported by BAIRD (1936), MOSSMAN (1937) and many other workers (cited in these two papers) but the problems of botanical microtechnique are more difficult and therefore further trials are needed before its use can be commended or rejected.

*Tertiary butyl alcohol:* Like dioxan it readily mixes with water, ethyl alcohol and paraffin. It solidifies at about 15°C and must therefore be kept at a temperature higher than this. The mixtures of water and TBA will however remain fluid unless the temperature goes down below 0°C. Dr. Donald A. Johansen (1937) recommends the following series:—

Solution No.	1	2	3	4	5	6	7	8	9	10
Water	95	89	82	70	50	30	15	0	0	0
Ethyl alcohol (95%)	5	11	18	30	40	50	50	45	0	0
Tertiary butyl alcohol	0	0	0	0	10	20	35	55	75	100
Ethyl alcohol (100%)	0	0	0	0	0	0	0	0	25	0
Percentage of alcohol (approximate)	5	11	18	30	50	70	85	100	100	100

Add some erythrosin to the last stages (No. 9) and make at least one further change with pure TBA.

The advantage of using TBA is that it does not dessicate the tissues, removing only the "free" and not the "bound" water. The tissues thus retain their property of absorbing water and can be softened after embedding in paraffin by soaking the blocks in water. For further information the reader is referred to Dr. Johansen's article (1937) from which the above summary has been prepared.

*Methylal or methylene-dimethyl-ether.* Like dioxan and tertiary butyl alcohol this too has the valuable property of being miscible with both water and paraffin. Full details regarding its use are not yet available. In a brief note DUFRENOY (1935) recommends that after washing out the fixative in water, the material should be transferred to a mixture of equal parts of water and methylal, then to pure methylal, and finally to a second methylal, dehydrated and neutralised by means of anhydrous sodium carbonate.

The device is time-saving and useful, but this reagent is expensive

and few have been successful with it. Further detailed information on the method of using it will be welcome.

### **The transfer from solvent to paraffin**

This should again be a gradual process otherwise much of the labour involved in the preceding stages will be undone. LAND (1915) has devised a way to avoid the paraffin resting directly upon the objects. WEATHER-WAX (1919) suggests a method of aerating paraffin by blowing cold air through it, thereby giving it a buoyancy sufficient to make it float upon the surface of the solvent.<sup>1)</sup> The writer uses GOODSPEED's method (1918). Some melted paraffin, which is at the point of solidification, is carefully poured down the sides of the vial containing the solvent and the material. Some of the paraffin immediately goes into solution but the rest will form a plug in the tube, which is now put away for a day or two (weeks and months will do no harm) till the solvent has become saturated with paraffin at room temperature. After this it should be placed on the top of the oven until the new saturation point is reached and then inside it, finally replacing the paraffin mixture with pure paraffin in the usual way.

With tertiary butyl alcohol and methylal the transfer is not made directly from the solvent to the paraffin, but in the first instance to a mixture of equal parts of paraffin oil and the solvent (see JOHANSEN, 1937; DUFRENOY, 1935), and then to pure paraffin.

### **Infiltration with paraffin**

Even if they are capable of maintaining a constant temperature, the defect with most paraffin ovens is that they are not well aerated and do not allow an easy escape of the fumes of the solvent. This necessitates a longer stay in the bath with consequent detriment to the material.

The simple old device of keeping the paraffin in a cylindrical vessel, heated from above by a carbon-filament bulb works very satisfactorily and is again coming into favour (MCCLUNG, 1937). A layer of paraffin remains unmelted at the bottom and since the material rests upon this, there is no danger of its getting overheated. The greater heat at the surface is an advantage since it hastens the evaporation of the solvent. If several lots are being infiltrated at the same time, there should be a correspondingly larger container for the wax and each lot enclosed inside a tea filter.

In places where neither gas nor electricity is available, vacuum flasks may be used for this purpose (RAU, 1929). The procedure is as follows:—nearly fill the flask with water at a temperature of 60°C; transfer the objects from the solvent (cedar oil was used by RAU) into

1) This is not necessary when chloroform is used as the solvent. The paraffin being lighter floats on its surface of its own accord.

melted paraffin contained in a short test tube; close the tube with a cork and insert it into the flask through a hole bored in the cork-stopper of the latter, pushing it down nearly as far as it will go; screw in the covering cap of the flask and leave, shaking the tube at intervals of 1-2 hours to facilitate penetration of the paraffin. The disadvantage of the method is that it does not allow any evaporation of the solvent which cannot therefore be got rid of by any other means except a frequent changing with fresh paraffin. However it still has some value if one is working in a small town or village where other means are not available.

Poor results may often be due to bad paraffin. Several makers supply a cheap grade which crumbles into powder on being pressed between the fingers. This is quite worthless for sectioning. HANCE (1933b) recommends the following method for improving paraffin:—

About 20 gms. of crude rubber, available in thin sheets, is chopped up and dropped into 100 gms. of smoking hot paraffin, stirring the mixture occasionally with a glass rod. In three to four hours the rubber melts and becomes completely mixed with the paraffin. To prepare a paraffin for embedding, the following are mixed and filtered while hot:—

Ordinary paraffin .....	100 gms.
Rubber-paraffin mixture .....	4-5 gms.
Beeswax .....	1 gm.

This yields a product which is pale yellow in colour and does not crystallise readily.

WESLEY (1928), in working on *Coleochaete*, heated the paraffin to the smoking point and kept it there for 6-7 hours in order to obtain a product of homogeneous texture.

The writer collects all discarded paraffin of the laboratory (containing xylol and other impurities), keeps it melted with a carbon filament bulb for a month or two and then filters it. This becomes slightly yellowish in colour but sections very well.

### Embedding

In tropical climates a paraffin of 56-58°C melting point is the most suitable but thin sections can be cut in winter from 52°C paraffin if it is ice cooled.

It needs considerable practice to arrange the objects quickly before the paraffin has solidified and the difficulty is greater in proportion to the smallness of the objects. FABERGÉ and LA COUR (1936) have described a method of using electrically heated needles to facilitate the work. In difficult cases when a longer time is required to arrange the objects in the desired plane, it is helpful to keep a small electric bulb over the embedding dish with a shade to protect the eyes from glare. This serves not only to light the objects but keeps the upper layer of the paraffin melted till such time as may be necessary. BUCK (1938) lowers a heating coil into the dish for the same purpose.

### The agar-paraffin method of embedding

Small objects are often difficult to handle by the usual methods and may get lost during the process of washing and dehydration. One method frequently employed by some workers is to wrap them in a piece of the inner epidermis from an onion scale or a small packet of animal membrane (previously killed and washed in water) and then carry the entire thing through the whole process, even embedding and cutting it under this cover. OHASHI (1930) used this method to cut sections of zoospores and sporelings of *Oedogonium*.

Following SCHWARZE<sup>1)</sup> (1922), BOLD (1933, p. 252) used the following method for his study of the cytology of *Protosiphon*. Material for fixation was grown on agar in petri dishes. Drops of the fixative were placed on the plants with a pipette and subsequently melted agar near the point of solidification was poured over the plants. The agar solidified almost instantly and small blocks containing the algae were cut out and dropped into vials containing a large quantity of fixative. These agar blocks with the included thalli were washed, dehydrated, imbedded and sectioned in the usual way.

The following procedure, suggested by MADGE (1936a) is capable of a wider application:—

1. Fix, wash, and run up the objects to 70% alcohol.
2. Make a 3% solution of melted agar and pour it on a warm slide to form a film 2.5 mm. thick. Quickly introduce the objects into this film and orient them as desired.
3. Allow the agar to set and cut the film into rectangular blocks containing the objects.
4. Dehydrate the blocks and proceed in the desired way to embed them, chloroform being preferred as an intermediary from alcohol to paraffin.
5. When trimming the blocks for sectioning, leave some paraffin around the agar. The agar, which remains on the slide after the paraffin has dissolved, remains quite transparent and does not interfere in the subsequent processes.

Miss MADGE (1936b) found this method to be invaluable for embedding the long styles of *Hedychium* so that they may be cut longitudinally without curling. After running up the fixed styles to 70% alcohol, a single style was laid on a slide wet with 70% alcohol and cut up into 8 mm. pieces. These pieces were next laid parallel to each other and in proper order on another slide coated with agar. Then blocks were cut out of the agar and embedded in the way already described.

The method employed by LA MOTTE (1937) for sectioning the megaspores of *Isoetes* is almost identical. Strips of heavy paper were attached to slides with rubber bands and then melted agar was applied with a small brush to form a layer 1–2 mm. thick near one edge of the

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1) HERRIG (1919) used an essentially similar method in his study of the pollen tubes of some angiosperms.

slide. The gametophytes were lined up in the desired way in furrows forged out of the agar with a flatpointed needle. Strips cut out of the agar were then removed and placed in the killing fluid. Alcohol was used for dehydration and a very graded series of xylols for clearing.

### Preparation of the sections

*The Microtome.* For quality as well as convenience in work, one needs two kinds of microtomes—one, a *rotary type* (the Spencer Precision Rotary Microtome will give life-long service) for serial sections of flower-buds, root-tips and most other objects that are not too hard, and another, a *sliding type* (the Jeffrey-Thompson microtome manufactured by the Bausch and Lomb Optical Works is probably the best) for cutting more difficult material. It is impossible to give any directions here on the use of the microtome; the best way is to watch an expert. There are many fine details which one learns to perform almost without consciousness and which so often make all the difference between success and failure.

*The Microtome Knife.* Knives intended to be used for microtomic purposes should be of the best steel available and rigorously tested with respect to their form and hardness. They are usually ground in two styles: *plano-concave* or hollow on both sides and *plain* or wedge-shaped with no such concavity. The former are satisfactory for cutting moderately hard objects only, but the latter are adequate for all purposes. Both types should be provided with detachable handles of convenient length which can be screwed in at the time of honing into the screw-hole at the back end of the knife. The plain type must have in addition some device to raise the back of the razor from the stone. This is accomplished by means of a tubular attachment which is to be slipped on the knife at the time it is being sharpened.

KISSER (1926) and UBER (1936) have described the methods of sharpening microtome knives. Prof. CHAMBERLAIN (1932) has given full directions on the methods of honing and stropping and they need not be recapitulated here.

The Lendvai apparatus, a case with 3 glass plates inside it, is of use for this purpose (LENDVAI, 1909). The three boxes of abrasives, supplied with it, contain respectively fine emery, prepared chalk and rouge. The knife is ground on the first plate with emery, made into a paste with water or olive oil. The same operation is repeated on the second plate with chalk and then on the third with rouge. If the whole process is carefully done, no stropping should be necessary.

J. A. LONG (1927) has devised a more elaborate sharpener described and commended by WETMORE (1932, p. 57) and UBER (1936).

*The use of safety razor blades in microtomy.* The obvious drudgery involved in the sharpening of microtome knives is responsible for the preference now given to safety razor blades. NESBIT (1926) and BHARGAVA (1934) have suggested a very simple method. Melt some hard paraffin on an old plain-surfaced knife and slip a clean blade upon it



in the desired position. The knife is now plunged in cold water and as the paraffin solidifies the blade sticks to the knife. NELSON (1935) uses "Duco Household Cement", and a couple of magnets hold the blade and knife together while the cement is setting. In either case, success depends on the rigidity of the contact between knife and blade and the correct distance to which the edge of the blade projects beyond the edge of the knife.

It is more convenient to use a regular blade-holder of which there are several types in the market (see CHAMBERLAIN, 1932; CRAIG and WILSON, 1935). Lack of success in its use may be due either to a defect in the holder so that it does not grip the blade rigidly, or to a mistake in the angle of inclination of the holder with respect to the block.

*Some difficulties in sectioning and how to overcome them.* Most of the troubles in sectioning common objects are due to imperfect infiltration or a dull razor. Three difficulties, not due to any of these causes, are dealt with below.

(1) It sometimes happens that the ribbon becomes 'electrified' during the process of cutting and sticks to the razor, scalpel, blade-holder or anything else brought near it in a most annoying manner, twisting and curling at the same time. It is to some extent related with atmospheric temperature and humidity but may be due to other reasons not clearly understood up to this time. Breathing upon the block or keeping a pan of boiling water near the microtome (WILSON & HOCKADAY 1935) will sometimes lessen the trouble. Another remedy is to stick in a piece of paper just below the razor and let the sections slide down the paper. This frequently effects some insulation. See also the methods devised by VARRELMAN (1938) and BLANDAU (1938). If none of them succeeds sectioning may best be postponed to some other time. If the same trouble recurs repeatedly, it might be worth while to repeat the embedding.

(2) In tropical regions it is very difficult to get good sections during the summer. Matters can be improved to some extent by ice-cooling the block and razor and maintaining a small chunk of ice over the former during the entire process of cutting. Further cooling may be brought about by keeping a block of ice on the platform of the microtome. CROSSMAN (1935) directs a stream of cold air over the paraffin block and HANCE (1937) has suggested a method for air-conditioning the microtome. Still another method of cooling the blade and the block is suggested by COOPER and MACKNIGHT (1937).

A ribbon can usually be obtained by any of the methods cited above, but when the temperature is very high (as in the month of May in certain parts of India) it sticks down immediately to the paper before one has time to cut it into suitable lengths and lift it for mounting on the slide. T. C. N. SINGH (1933) suggests floating the ribbon on cold water where it can be cut by means of a pair of scissors.

(3) When a block fails to yield coherent sections even after thorough infiltration, it should not be thrown away but left in a pan of water for a week or so. Paraffin has the property of absorbing small quantities of water and after such treatment many objects yield good

sections, though they would hardly cut without it (LAND, 1915). It is possible to hasten the penetration of water by trimming away some of the paraffin around the block and expose that side along which the sections are going to be cut. The writer was able to cut such hard and thick stems as those of *Boerhaavia* by this device. The methods employed by COUCH (1930) and CUTULY and CUTULY (1934) are based upon the same idea. Sometimes, however prolonged soaking may be injurious. Softer tissues frequently tend to become mushy and may even crumble into a powder when the razor is passed through the block.

*Fixing sections to the slide.* Meyer's albumen is in general use for this purpose, but does not always hold thick sections of pine cones, moss capsules, *Selaginella* strobili and other refractory objects.<sup>1)</sup> SZOMBATHY (1918) recommended gelatin and HAUPT (1930) has published a modification of this formula which is very satisfactory. The writer and his pupils use Land's fixative (LAND, 1915), substituting "gloy" in place of gum arabic (MAHESHWARI, 1929). About 5-6 drops of gloy should be used to make 20 cc. of the fixative. Exact proportions cannot be given since different makes of "gloy" vary in thickness and even the same make becomes much thicker towards the end than when it is fresh. If too much gloy is used, the sections will of course stick well, but the background shows a disagreeable colouration. If too little is used, the sections will come off in water.

### The staining and mounting of the sections

An excellent account of the history of the use of dyes in microscopy has been given by KORNHAUSER (1930) and CONN (1936). Most of our present day staining processes were devised and perfected by some biologists of the last century, among whom Flemming, M. Heidenhain, Strasburger, Ehrlich and Benda deserve special mention. It is not necessary to go into these methods in detail, for they have been repeatedly described in the standard texts on this subject. It will be enough here to call attention to a few combinations which have come into use recently or in which notable improvements have been made during the last decade.

*Preparatory treatment.* After the paraffin has been dissolved out from the sections and they have been brought down to 50% alcohol or water, they are ready for staining. No further preparatory treatment is needed except in the case of material fixed in a fluid containing osmic acid. There are several ways of bleaching out the black colour, e.g., by the use of hydrogen peroxide, chlorine water, sulphurous acid, or potassium permanganate and oxalic acid. The first is the most convenient. Add about 4-6 cc. of Merck's "Superoxol" to 60 cc. of 95% alcohol and let the slides remain there till the black colour is no longer visible under

1) SCHNEIDAU (1937) recommends the following solution:—white of one egg; glycerine 50 cc.; sodium salicylate 1 gm.; distilled water 1500 cc.; mixed thoroughly and filtered. The mixture is placed in a bottle with rubber teat and run on the slides with the sections floated and warmed in the usual way.

the microscope. This may take one to several hours. The action is quicker on exposure to light. A long immersion (8–12 hours) of the slides in 1% chromic acid may also serve the same purpose and has the further advantage of acting as a mordant for some stains.

*Safranin and fast green FCF.* Safranin is one of the most valuable stains known to the histologist both for nuclear as well as anatomical and embryological studies. Till a few years ago a particularly favourite combination used by many botanists was that of safranin and light green. Its greatest drawback is that the light green is very fugitive and after two or three years the green colour may disappear entirely. HAYNES (1928) advocated the use of fast green FCF which had come into use as a food dye. We have used it ever since on a great variety of plant material. Several thousand slides have been prepared during the last ten years and the stain is still bright in all of them. The writer regards this combination as so satisfactory that for routine work he and his pupils have practically given up the use of Delafield's haematoxylin. The following schedule is recommended:—

1. Bring sections down to 50% alcohol.
2. Stain in a 0.5% solution of safranin 0 for 3–12 hours. The solution can be made up in water or 50% alcohol depending on the kind of safranin used.
3. Rinse slides in water and treat very briefly with acid alcohol (a few drops of HCl in 50% alcohol). TUAN (1930) recommends an alcoholic solution of picric acid.
4. Wash thoroughly in water.
5. 50, 70 and 95% alcohols.
6. Treat with a 1½% solution of fast green in absolute alcohol, moving the slide constantly. The time will generally vary from 1 to 5 minutes but must be determined by experimentation with each object.
7. Pure absolute alcohol.
8. Xylol.
9. Mount in balsam.

Chromosomes, nucleolus and lignified walls show a bright red colour, while cellulose walls and the cytoplasm will be stained green. The treatment with acid alcohol may be shortened or even omitted if the red colour escapes too readily.

The procedure recommended by MOORE (1936) is essentially similar to that which we have been using with the difference that he interpolates a clove oil stage between absolute alcohol and xylol. We think that this has no advantage unless the absolute alcohol contains too large a percentage of water to interfere with the clearing of the sections in xylol.

*Crystal violet and Erythrosin B.* This is a very useful combination, mainly employed for anatomical studies. It can be used with greater success than safranin and fast-green on herbaceous stems and roots and parts of aquatics, where the lignification is not sufficiently pronounced. The following schedule has been repeatedly tried by us on various objects and can be relied upon to give successful results, particularly if the material has been fixed in a fluid containing chromic acid:—

1. Bring sections down to water.
2. Stain in a 1% aqueous crystal violet for 10–20 minutes. This gives a deep blue colour; where a redder shade is required, methyl violet 2B should be used in an exactly similar way.
3. Rinse in water and rush up through 50 and 90% alcohols.
4. Treat with 1% erythrosin or orange G dissolved in absolute alcohol.<sup>1)</sup> Only a brief application is necessary.
5. Rinse with fresh absolute alcohol.
6. Differentiate with clove oil, watching under the microscope till the desired intensity of the two stains is reached. If the crystal violet is feeble, bring the sections down to water and begin again. If the erythrosin or orange G is too little, this can still be remedied by flooding the slide with a clove oil solution of the same dye, previously kept ready for use.

Lignified walls stain blue and cellulose walls take up the colour of erythrosin or orange G.

*Crystal violet and iodine.* During recent years crystal violet has found greater application in karyological studies. Due to its transparency, even thick sections (with uncut nuclei) can be clearly stained with it, enabling more accurate chromosome counts. It is also superior to haematoxylin for studies on chromosome structure, but all material intended to be stained by this method should be fixed in a fluid containing chromic acid. The following schedule, known as Newton's method, is the one most widely used at present (LA COUR, 1937) :—

1. Bring slides down to water.
2. Stain 3–10 mins. in a 1% aqueous solution of crystal violet, boiled and filtered.
3. Rinse in water.
4. Treat with Lugol's solution (1% iodine + 1% KI in 80% alcohol) for 30–45 seconds.
5. Rinse in 95% alcohol.
6. Absolute alcohol.
7. Clove oil, differentiating under the microscope.
8. Xylol, 2 or 3 changes at intervals of 5 mins. each to make sure that the clove oil has been removed completely.

Smith (1934) claims to have found a method of making this stain as permanent as iron-haematoxylin and recommends the following procedure :—

1. Run down slides to 95% alcohol.
2. Treat with Lugol's solution for 10–20 minutes.
3. Rinse in water.
4. Stain in crystal violet, boiled and filtered before use.
5. Rinse in water.
6. Rinse in a second jar of Lugol's solution.

1) JACKSON (1926) uses it in clove oil. From our experience we have come to the conclusion that clove oil solutions are generally less preferable since they do nothing more than give a "paint" to the tissues on which they are used.

7. Rinse in 95% alcohol.
8. Flood with sat. sol. of picric acid in absolute alcohol.
9. Wash immediately with pure absolute alcohol for a few seconds.
10. Flood slide with clove oil to differentiate.
11. Xylol.
12. Balsam.

This method of staining gives a clear yellow cytoplasm and purple chromosomes. G. O. COOPER (1936), BEARD (1937) and MACKAY (1937) are among those who report very satisfactory results with this method.

*Safranin-crystalviolet-orange G.* This combination, proposed by W. FLEMMING (1891), has all along been very popular with us. MARGOLENA (1935) has recently published a revised schedule, interpolating the use of Lugol's solution to stabilise as well as hasten the differentiation:—

1. Stain  $1\frac{1}{2}$ –3 hours in  $\frac{1}{2}$ –1% safranin.
2. Rinse in distilled water.
3. Treat with Lugol's solution for 15–25 seconds.
4. Rinse in distilled water.
5. Stain 5 mins. in a 1% aqueous solution of crystal violet.
6. Rinse in distilled water.
7. Treat again with Lugol's solution for 3 seconds.
8. Absolute alcohol.
9. Differentiate with clove oil and orange G.
10. Xylol.
11. Balsam.

Steps 3 and 4 may be omitted if the material has been fixed in Navashin's fluid.

*Iron-alum haematoxylin.* Among all modern cytological stains this is still used most widely. The process consists of 3 steps: mordanting, staining and differentiation.

The Mordant: A 2% or 4% solution of iron-alum (Ferric ammonium sulphate) is used for this purpose. Only the pure violet crystals should be selected for the solution. This salt is difficult to keep in hot climates as the crystals become covered with a brownish coating and melt in their own water of crystallisation. Those who have access to a refrigerator should keep the bottle inside it, tightly closed with a rubber stopper. Various substitutes have been tried but without any appreciable success. LANG (1936) has recently recommended the following mixture:—

4% Ferric ammonium sulphate .....	500 cc.
Acetic acid .....	5 cc.
Sulphuric acid .....	0.6 cc.

This is said to have better keeping qualities and to remain clear for a considerable length of time without giving the disagreeable precipitates so common with the sulphate alone. The acetic acid mordants the stainable structures more vigorously and obtains a greater specificity of the stain but may be omitted if a purple tone is preferred to black in the

finished preparation. The trace of sulphuric acid prevents oxidation of the ferric ammonium sulphate. The time of mordanting should be 30 mins. to 1 hour if the iron salt is used alone but 2-12 hours in the case of Lang's solution.

**The Stain:** For staining a  $\frac{1}{2}\%$  solution of haematoxylin is prepared either by dissolution of the dry stain in a small quantity of alcohol and then adding the requisite quantity of water or by pouring hot water directly over the stain. We prefer the latter course and use tap water for this purpose. If distilled water is used, it is worth while to add a small quantity of sodium bicarbonate to the solution (see also CAMP, 1931; HANCE, 1933a).

It was customary in the past to let the solution ripen for a month or so. In the tropics ripening as well as deterioration of the stain take place much faster than in the colder climate of Europe. KOHL and JAMES (1931) ripen the solution in a very short time by exposing it in a shallow dish to a powerful quartz mercury vapour light and stirring it frequently. At present, however there are several haematoxylin solutions on the market which ripen in a very short time and can be used only 2 or 3 days after their dissolution in hot water.

The time of staining should be about 30 mins. to 2 hours. The modern tendency is to shorten the period of mordanting as well as staining to the minimum.

**Differentiation:** When the slides are taken out of the stain they show a uniform black colouration. Differentiation is carried out either by means of 1-2% iron-alum, or by an aqueous solution of picric acid (TUAN 1930; MAHESHWARI 1933), or by the following mixture proposed by LANG (1936): 2% iron alum 500 cc; acetic acid 5 cc; sulphuric acid 0.3 cc. The advantage of using picric acid is that it primarily destains the cytoplasm and leaves the chromosomes practically unchanged. After mounting in this way the chromosomal image is clearly defined on a transparent cytoplasm without the disagreeable brownish colourations and precipitates which frequently appear after the use of iron-alum.

In all cases destaining should be carefully watched under the microscope and in more critical work under a water immersion lens. JEFFREY (1937) thinks that for chromosome studies the haematoxylin should be almost bleached out with iron-alum and then followed by aqueous safranin overnight. This is largely removed in the process of dehydration, "but enough remains in the nuclear structures to support a richness of detail not attained by haematoxylin".

After differentiation the sections should be thoroughly washed in running water, then dehydrated through the alcohol series, cleared in xylol and mounted in balsam in the usual way.

**Feulgen reaction.** Although a valuable stain, haematoxylin has the disadvantage of blackening too many structures and providing little or no differentiation between chromatin, nucleolus and various extra-nuclear bodies. FEULGEN and ROSSENBECK, in 1924, devised the following specific reaction called "Nuclearreaktion" by which only the chromatin is stained. It is based upon the use of a decolourised form of basic fuchsin which works

selectively and reacts with chromatin to give a purple colour. According to the original instructions of FEULGEN and ROSSENBECK (1924) fixation should be carried out in a fluid containing corrosive sublimate; but YASUI (1933) used ethyl alcohol as the fixative, and CARLSON (1936) tried several different fixing fluids, all of which were successful.

The following schedule for staining the sections has been adapted from de TOMASI (1936) :—

1. Bring slides down to water.
2. Treat for 2 mins. with cold normal HCl and then for 4–20 mins. with the same raised to a temp. of 60°C. Rinse again in cold normal HCl and then in distilled water. The optimum time of hydrolysis (this breaks up the thymonucleic acid in the chromatin and liberates aldehydes which later give a purple colour with the fuchsin sulphurous acid used in step no. 3) depends on the particular object and may also vary with the fixing fluid. Failure to obtain a satisfactory stain may depend on hydrolysis for too long or too short a period.
3. Stain in fuchsin sulphurous acid for 2–5 hours till the sections show a definite purplish tinge. To prepare the stain, pour boiling distilled water (200 cc) over 1 gm. of basic fuchsin<sup>1)</sup> and shake vigorously; cool to 50°C; filter and add 20 cc. of normal HCl to the filtrate; cool further to 25°C and add 1 gm. of anhydrous potassium metabisulphite. Cork well and put in a dark place. Within 24 hours the solution is decolourised to a light straw or faint pink colour and is ready for use. It should remain stored in a dark place.
4. Pass slides through three successive baths of the following solution allowing them to remain for 10 minutes in each:—

Distilled water .....	200 cc.
10% aq. sol. of potassium meta-bisulphite ....	10 cc.
Normal HCl .....	10 cc.

5. Rinse in distilled water.
6. Run up through the alcohol series, counterstaining if necessary with alcoholic fast green or orange G.
7. Xylol and balsam.

MILOVIDOV (1936), who has tried the Feulgen method on many plants says that it is invariably successful and that the negative findings of other workers are due to poor technique or to the presence of resin, tannin, slime etc, in the cells. His paper is of great value to all cytologists who have occasion to use this reaction.

### Conclusion

Variations in all stages of the processes outlined above will suggest themselves to any investigator working in this line. Some of them will be valuable and may lead to further improvements in the technique. The

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1) MARGOLENA (1932) recommends Grüber's fuchsin f.B. and Coleman & Bell's fuchsin C.F. 10. See also SCANLAN & MELIN (1937).

following are the three greatest needs of the paraffin method at the present time:—

1. A fixing fluid that will penetrate more quickly than the one devised by Navashin, but be as efficient as this in other respects.
2. A dehydrating agent that will mix freely with water, alcohol and paraffin. Dioxan, tertiary butyl alcohol and methylal have been proposed for this purpose and their possibilities should be fully explored in the future.
3. A stain that will be as permanent as iron-haematoxylin but more transparent than this, so that it can be used equally well on thin as well as thick sections.

In conclusion, I must express my warmest thanks to my wife and sister for the valuable help that they gave me in trying many of these processes and to many colleagues and co-workers throughout the world who exchanged botanical specimens and slides with me and thereby helped me in an indirect manner to continue my enthusiasm for the field of plant microtechnique.

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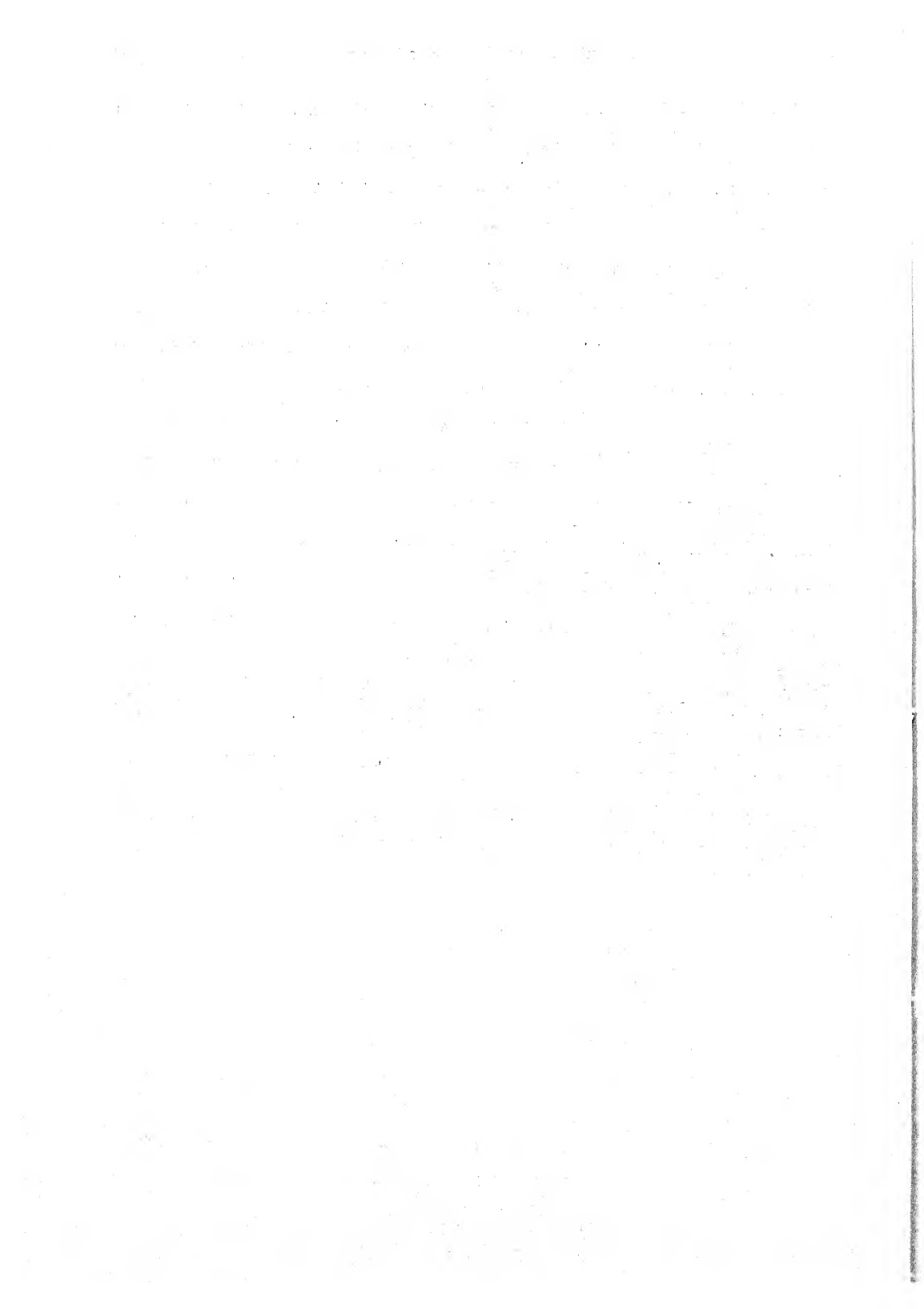
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## On the Chromocentre Observed Through the Mitotic Cycle of Somatic Cells in *Drosophila virilis*<sup>1)</sup>

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(With 13 Figures in the Text)

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It is now a well established fact that, the chromosomes in the nuclei of salivary glands of *Drosophila* are all connected together by their proximal ends to a deeply staining chromatic region which was designated by Heitz ('33a, '33b) as the chromocentre. Along with the study of the salivary gland chromosomes themselves, much attention has been devoted to the origin and structure of the chromocentre both from the morphological and genetical stand-point. That the chromocentre is built up through the fusion of inert chromatin lying adjacent to the spindle attachment regions of the contributing chromosomes, is a view which is now generally held, supported by recent studies which have correlated cytological observations with genetical criteria. There is, however, at present no uniformity of opinion regarding the structural features of the chromocentre of the salivary gland nucleus and different views have been put forward regarding this subject by a number of investigators (Heitz '33a, '33b, Painter '35, Painter & Stone '35, Koller '35, Prokofyeva-Belgovskaya '35, Yasui '35, Frolova '36, Bauer '36, Emmens '37, Kaufmann '37).

That there exists in mitotic nuclei of *Drosophila* a deeply staining chromatic body, which in its nature seems to be comparable with the chromocentre of the salivary gland nucleus, has been shown by the studies of Heitz ('33a, '33b) and Frolova ('38). Heitz ('33a, '33b) suggested that the chromocentre of the salivary gland nucleus represents the heteropycnotic regions of the mitotic chromosomes. As a prerequisite to the elucidation of the chromocentre in the salivary gland nucleus, a precise knowledge regarding the structure and behaviour of the chromocentre in the mitotic cells is highly desirable since it may yield critical information and prove helpful in interpreting that structure in the salivary gland cell. The present paper dealing with the behaviour of the chromocentre

1) Contribution No. 138 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo, Japan.

during the mitotic cycle in the cells of the oesophageal ganglia of the larvae of *Drosophila virilis* has been prepared with this point in view.

Wild-type flies of *D. virilis* from the same stock as the material of the previous study (Makino '38) were employed in this investigation. Temporary smears were made by the aceto-carmin technique. Ganglia were removed from the larvae about to pupate in isotonic salt solution on a clean glass slip and placed in a watch crystal containing aceto-carmin for 1-2 hours. Reference may be made to the previous paper as regard the whole procedure for making these preparations. In some special cases, the double staining method of iron-haematoxylin with light-green and the Feulgen reaction were employed for the sectioned material.

The drawings were made with an Abbe camera lucida using a Zeiss 2 mm. apochromatic objective and a compensating ocular K 20  $\times$ , giving a magnification of 2400 diameters.

The author is greatly indebted to Prof. K. Oguma for his valuable advice in the course of this work.

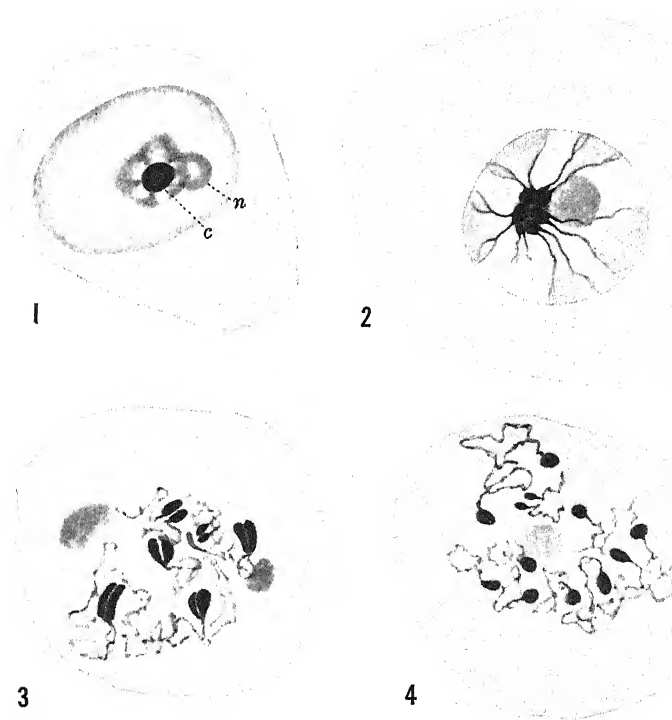
### Observations

Interphase nuclei show a more or less indefinable reticulum and two prominent bodies of an irregularly spherical form (Fig. 1). One of them is a deeply staining compact body entirely chromatic in appearance (Fig. 1, *c*). This body always occupies the central area of the reticulum, close to the periphery of the nucleus. The other is a less stained but sharply outlined body achromatic in nature. It lies in the nuclear cavity, always in close proximity to the former body (Fig. 1, *n*). On comparing its characteristics with the nucleolus seen in the salivary gland nucleus and also noting its staining reaction as stated below, this body can be no other than the plasmosome or true nucleolus.

When subjected to the double staining method with iron-haematoxylin and light-green, the chromatic body (*c*) was intensely stained with haematoxylin while the nucleolus (*n*) with light-green. When tested by the Feulgen method, only the chromatic body gave the characteristic reaction of chromatin.

As the prophase advances, the reticulum is entirely resolved and there appears in its place a series of six sharply defined pairs of chromatic strands as shown in Fig. 2. These strands fuse at their proximal ends to form the central chromatic body. Each pair consists of two distinct threads lying side by side. The number of these pairs just corresponds to the haploid chromosome number of this species and the total number of threads represents the number

of the diploid chromosomes. From the above evidence there is no doubt that the members of each pair represent homologous elements. Five pairs are extremely long, and the distal ends of all the threads are longitudinally split, their extremities becoming almost indefinable. The members of the 6th pair of threads are conspicuously short in length as compared with the others. A prominent nucleolus occurs in close proximity to the central body. Here a pronounced similarity in configuration is seen between such a prophase nucleus of the mitotic cell and the nucleus of the salivary gland cell, as emphasized in the previous paper (Makino '38). At the same time,



Figs. 1-4. Prophases, neurocytes of larvae of *Drosophila virilis*. 1, interphase, ♀. c, chromocentre. n, nucleolus. 2, early prophase, ♀. Six pairs of threads fuse at their proximal regions into the central chromatic concentration (chromocentre). 3-4, early prophases, ♀. The nucleus is crushed to show the components of the chromocentre. For explanation, see text.  $\times 2400$ .

it becomes easily noticeable that the central body of the prophasic nucleus to which six pairs of chromatin threads converge, corresponds in its structural outline to the chromocentre of the salivary gland nucleus. Heitz ('33b) also found a similar body in the resting nucleus of *D. virilis* and that of some other species, describing it as 'Chromozentrum'.

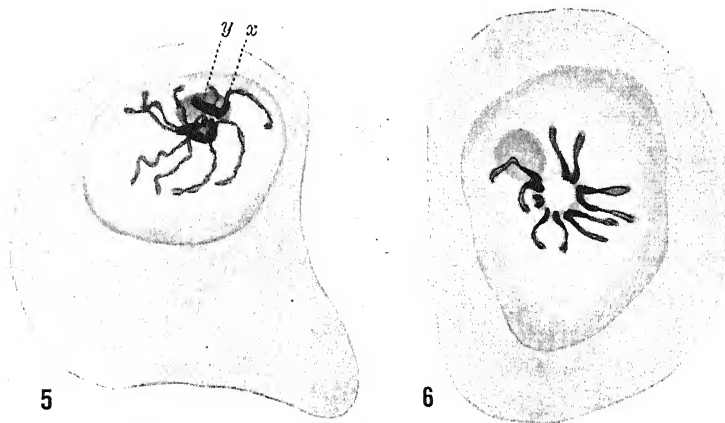
In the early stage of prophase the chromocentre appears as a compact concentration as already mentioned. In the advanced stage however, the chromocentre does not appear as a single homogeneous body but shows a differentiation into several smaller bodies. A close examination of such a stage discloses that six differentiated bodies, five large and one small, are found included in the chromocentre; and that from every one of these six bodies a pair of chromatic threads seem to emerge (Fig. 2). In other words, every two homologous threads come into contact at their proximal ends with a common small body. In this state, therefore, the chromocentre may be regarded as an aggregate of six smaller bodies, from each of which a pair of threads is being developed. This fact is further verified by the following evidence. When such a nucleus is crushed by pressure applied to the cover glass, the chromocentre segregates into six component parts as demonstrated in Fig. 3. As seen in this figure, each of these six bodies is longitudinally split and to each of them a pair of elongated chromatic threads are attached. If further pressure is applied to the cover glass, they finally separate into their composing halves and thus twelve bodies are observed, each united with a single thread (Fig. 4). According to Heitz<sup>1)</sup> ('33b) the proximal chromatic regions in prophasic chromosomes are composed of heterochromatin and the distal thread regions consist of euchromatin. Hence the following conclusion is warranted that in the mitotic nucleus of *Drosophila virilis* it is these heterochromatic regions forming the proximal portions of chromosomes which fuse to form the chromocentre.

Later as the prophase progresses the thread parts, the euchromatic regions of chromosomes, become gradually shortened probably due to the spiralization of the chromonemata and assume a well defined appearance (Fig. 5). At this time the highly condensed structure of the chromocentre is lost from view. The threads arrange themselves in a radial manner emerging from the central area where their proximal heterochromatic parts aggregate. In a more advanced stage, the threads become shorter and thicker, and longitudinal splitting is distinctly visible in each. These threads lie in pairs side by side and twelve individual elements make their well-defined appearance (Fig. 6).

1) Heitz ('33a, '33b) pointed out that the chromosomes of *Drosophila* are longitudinally differentiated as regards staining reaction into two kinds of chromatin—heterochromatin and euchromatin; and he further postulated that the heterochromatic portions which fail to undergo differentiation and remain chromatic through interphase, are genetically inert or gene-less. In *D. virilis* the proximal halves of chromosomes are heterochromatic in structure and the distal halves euchromatic, with the exception of the Y chromosome which is totally heterochromatic.



A noteworthy feature as the nucleus is going through these stages is the evidence that there exists a remarkable distinction in the chromosomal configuration between the male and female cells. In the male cell, there occurs an elongated chromatic element which is not accompanied by any thread at its distal region and which contributes to the formation of the chromocentre together with the heterochromatic parts of other elements (see the elements indicated as *y* in Fig. 5); while in the female cell no such element is found (see Fig. 6). That this element is no other than the Y chromosome is indisputable in view of well established evidence obtained through



Figs. 5-6. Later prophases, neurocytes of larvae of *D. virilis*. 5, ♂: *x*, the X chromosome. *y*, the Y chromosome. 6, ♀.  $\times 2400$ .

the work of Heitz ('33b). In Fig. 5 there is found an element the heterochromatic region of which is associated with the Y. There is no doubt from its position that this element represents the X (*x* in Fig. 5).<sup>1)</sup> According to Heitz, the Y element is totally heterochromatic possessing no euchromatic part. It is highly probable that the Y retains its condensed form in the resting nucleus and contributes to the formation of the chromocentre together with the heterochromatic regions of the other elements. In the prophase such as shown in Fig. 2, the Y seems to maintain its condensed appearance in the male cell, without developing any thread from its distal part as the others do, though a conclusive statement regarding

1) As is already shown by the admirable work of Heitz ('33b), in *Drosophila* the chromosomes concerned with the nucleolus-formation in prophasic cells are the sex chromosomes; in the male they are represented by the X and Y, while two X's represent these in the female. A detailed account of our observations on this subject will be given elsewhere with some additional evidence relating to the nucleolus-chromosome relationship observed in the giant somatic cells.

this cannot be made because of the complicated structure of the chromocentral region in such a stage. But there is some evidence to support this statement. In the interphasic nucleus of the male cell there is occasionally found a peculiar configuration in which the Y alone lies beside the chromocentre being partially, not entirely embedded in the latter (Fig. 7).

In the early metaphase, the most striking characteristic of the nucleus is that all the chromosomes become free from the proximal

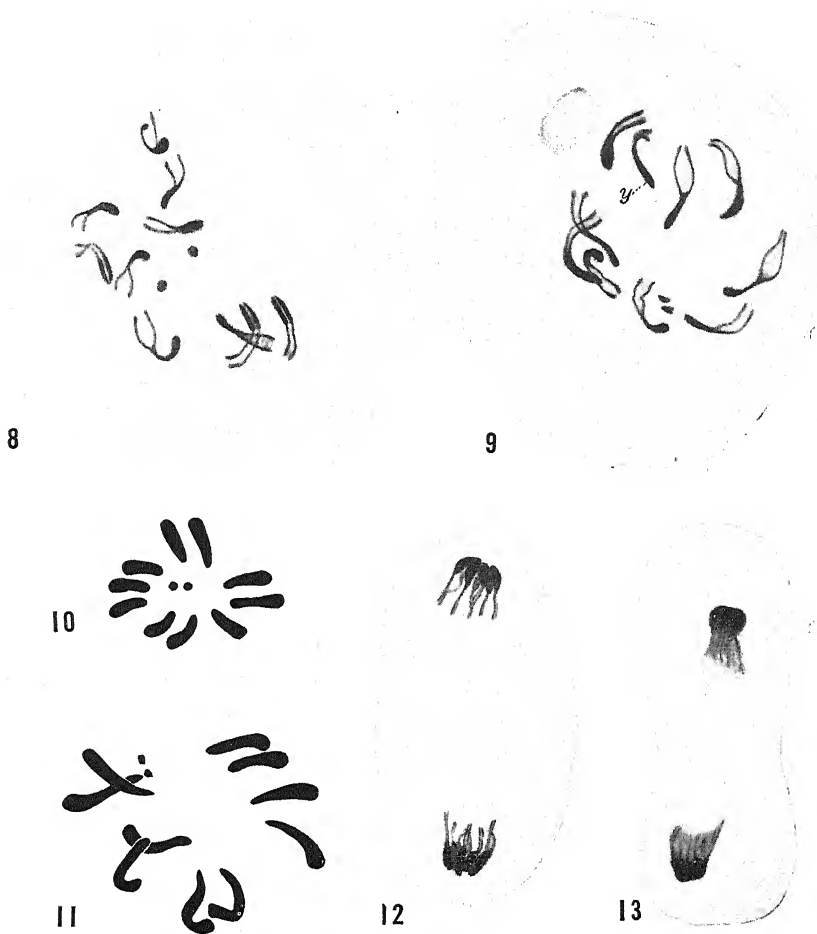
aggregation of their heterochromatic regions and lie scattered in the nuclear cavity (Figs. 8-9). The male cell is easily distinguishable from the female one because in it the Y element persists in its totally heteropycnotic condition in striking contrast to the other elements, all of which being composed of two differentiated parts, heterochromatic and euchromatic (Fig. 9). All the chromosomes, barring the Y and those forming the smallest pair, show at this stage a differentiation into two kinds of chromatin along their longitudinal axis. Their proximal halves are composed of compact heterochromatin and the distal ones made up of diffusely stained euchromatin.

In this condition, therefore, the chromosomes clearly exhibit in their approximately median part<sup>1)</sup> a line of demarkation where their two portions—heterochromatin and euchromatin—join each other, without showing any variation in all the different cells observed. The longitudinal splitting is quite distinct in every chromosome along its long axis. The smallest pair looks like being totally heterochromatic, but this is not actually the case, as, in some cases, one of their extremities gives evidence of being composed of a fine thread, which suggests the existence of an euchromatic part.

1) In this respect Heitz ('33b) describes as follows: "Auch bei *D. virilis* läßt sich nicht vollkommen genau angeben, wie groß das heterochromatische Chromosomenstück ist. Soviel man aber auch den Metaphasen erssehen kann, scheint es eher etwas größer als die Hälfte zu sein."

Fig. 7. Interphase, ♂, neurocyte of larva of *D. virilis*. The Y element is distinguishable lying near the chromocentre, not entirely embedded in the latter.  $\times 2400$ .

At the metaphase when the chromosomes take a radial arrangement on the equatorial plate, the structural differentiation into heterochromatic and euchromatic parts disappears altogether in every chromosome, every one of them being uniformly stained giving a smooth outline (Figs. 10–11). Under such a condition, therefore, there exists no distinction in the chromosome complement between the male and the female, due to the lack of any visible distinction between the X and Y. The chromosome complexes of both the sexes are similar, twelve well defined chromosomes being observable in either of them (compare Fig. 10 and Fig. 11). The homologous



Figs. 8–13. Late prophases, metaphases and telophases; neurocytes of larvae of *D. virilis*. 8, late prophase just before the metaphase arrangement, ♀. All the elements are longitudinally differentiated into two kinds of chromatin—hetero- and euchromatin. 9, late prophase, ♂. The Y chromosome (*y*) is totally heterochromatic. 10, metaphase, ♀. 11, metaphase, ♂. 12, telophase, ♀, showing the differentiation of chromosomes into hetero- and euchromatin. 13, late telophase, ♀.  $\times 2400$ .

mates lie side by side in close proximity to each other, a remarkable condition commonly seen in the Diptera in general.

After getting crowded at the pole in the telophase following the anaphase segregation, the chromosomes again show a distinct differentiation into two structural components in each, probably excepting the Y. Their proximal halves remain aggregated, persisting in their deeply staining heterochromatic condition while their distal halves are converted into diffusely staining euchromatic threads (Fig. 12). Later the heterochromatic parts of all chromosomes become fused together to form a dense compact body, the chromocentre, and, at the same time the euchromatic parts disintegrate into the reticulum, thus forming two new interphasic nuclei (Fig. 13).

### Remarks

It is shown in the works of Heitz ('29, '32) that in the resting nuclei of many plants there are chromatin granules or aggregations which remain chromatic through the interphase, this condition being known as heteropycnosis. Heitz pointed out in his papers that in many plant chromosomes there is a longitudinal differentiation of the chromatin as regards staining reaction and that those parts which are deeply stained fail to undergo disintegration in telophase and remain fully formed in prophase. The true chromatin which undergoes differentiation in telophase is designated by him as *euchromatin* and the substance composing the heteropycnotic portions as *heterochromatin*.

A similar condition is sometimes be found in the nuclei of certain animal cells. Eisen ('00)<sup>1)</sup> and Janssens ('05)<sup>2)</sup> described such a phenomenon in spermatogenetic stages of the salamander, *Batrachoseps*. Recently Corey ('38) observed that in several species of grasshoppers (Acrididae), chromosomes of the male germ cells possess deeply staining heteropycnotic polar granules which persist as the heteropycnotic bodies from the spermatogonial telophase to the succeeding growth stages. Many other organisms also show a polarization during meiosis (zygotene) and this is often regarded as depending on the presence of heteropycnotic regions in the chromosomes. Thus the presence of heteropycnotic regions in the chromosomes, which fuse to form during the interphase the chromatic concentration such as the chromocentre in Diptera, is not an uncommon phenomenon.

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1) Journ. Morph., 17.

2) La Cellule, 22.

The study of Heitz ('32) above quoted, presents, furthermore, a discussion of the functional differentiation of two kinds of chromatin—euchromatin and heterochromatin—and postulates that the heteropycnotic portions contains few genes. In his further study on several species of *Drosophila* Heitz ('33b) correlates conditions in salivary gland nuclei with those of mitotic nuclei, and, in conjunction with evidences of gene distribution obtained from genetical experiments in *Drosophila melanogaster* by Muller and Painter ('32) and others, he confirmed his previous concept that there exists a relationship between the visible, structural longitudinal differentiation of chromosomes into euchromatin and heterochromatin and invisible genic differentiations whereby the heterochromatic portions would be inert or gene-less.

In *Drosophila virilis*, as pointed out first by Heitz ('33b) and later in the present study, there is seen a distinct, structural longitudinal differentiation into two kinds of chromatin in every one of the chromosomes excepting the Y. The proximal halves of chromosomes, adjacent to the spindle attachment regions are composed of heterochromatin and their distal halves are made up of euchromatin, while the Y is totally heteropycnotic. Relying upon the evidence established by him in *Drosophila melanogaster*, Heitz ('33b) suggested that in *Drosophila virilis* too these heteropycnotic portions forming the proximal halves of autosomes and of the X as well would be genetically inert, though at that time he could not correlate cytological observations with genetic criteria. Recently Fujii ('37) has reported that the above-mentioned assumption of Heitz seems to be correct on the basis of his findings from experiments of the gene location obtained through induced translocations. That in mitotic nuclei it is these proximal heterochromatic regions which fuse, including a part of the Y chromosome, to form the chromocentre, was first suggested by Heitz ('33b) and this has been ascertained in the present study by following the behaviour of the chromocentre through the mitotic cycle.

In conjunction with studies on gene distribution recent investigations of the salivary gland chromosomes in *Drosophila* have emphasized that the chromocentre is built up through the fusion of inert chromatin (heterochromatin) devoid of genes, lying adjacent to the spindle attachment regions of the contributing chromosomes. The investigations of Prokofyeva-Belgovskaya ('35), Yasui ('35), Frolova ('36), Bauer ('36), Emmens ('37) and Kaufmann ('37), all seem to show that the chromocentre of the salivary gland nuclei of *Drosophila* is entirely chromosomal from the morphological viewpoint. Heitz ('34), in his work on salivary gland chromosomes of

*Drosophila virilis*, has found two kinds of heterochromatin which form the chromocentral region. According to him the central-most part of the chromocentre having a compact appearance is called  $\alpha$ -heterochromatin and the other is  $\beta$ -heterochromatin which lies immediately adjacent to the former at the proximal ends of four out of the six chromosome strands. At present it can not be stated with certainty as to which parts of the chromocentre seen in the resting mitotic nucleus the  $\alpha$ - and  $\beta$ -heterochromatins forming the chromocentre of the salivary gland nucleus correspond. However, the existence of a pronounced similarity found between the essential structure of the salivary gland nucleus and that of the resting mitotic nucleus, as already emphasized in the previous paper (Makino '38), is significant and leads us to believe that the general structural condition of the chromocentre of the salivary gland nucleus is very likely in agreement with that of the mitotic nucleus. As seen in Fig. 2 in this paper, in the resting stage of the mitotic nucleus of *Drosophila virilis* there appear six pairs of chromatic threads, all converging at their proximal ends to the fused body. That these six pairs of threads correspond to the six strands of the salivary gland nucleus and that the central body from which the threads diverge is identical to the chromocentre of the salivary gland nucleus, has become evident from the present study. It was pointed out by the author (Makino '38) that the salivary gland nucleus and the other somatic giant nuclei of larval cells as well can be regarded as existing in a permanent resting stage condition in which the nucleus has undergone tremendous growth without the accompanying nuclear division. Hence the following assumption is warranted that in the course of growth the active parts of chromosomes, which are composed of euchromatin exclusively, develop into the characteristic huge spireme-like chromosomes with cross-striations, while their inert portions consisting of heterochromatin and forming by fusion the chromocentre, remain in the initial condition without showing any sign of enlargement.

### Summary

The mitotic chromosomes of *Drosophila virilis* observed in the oesophageal ganglion cells are longitudinally differentiated as regards the structure into two kinds of chromatin—heterochromatin and euchromatin. The proximal halves of chromosomes adjacent to the spindle attachment regions which are composed of heterochromatin remain chromatic and fail to disintegrate during the interphase, while the distal halves consist of euchromatin, with the

exception of the Y chromosome which is totally heteropycnotic. Observations made through the mitotic cycle showed that it is those heterochromatic regions which fuse, including the Y chromosome, to form the chromocentre right through the telophase to prophase. Some remarks on the relation between the chromocentre of the salivary gland nucleus and that of the mitotic nucleus are given.

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## An Abnormal Staining Capacity of the Sixth Salivary Gland Chromosome of a Strain of *Drosophila virilis*

By

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### Introduction

In the course of my studies on the salivary gland chromosomes of *Drosophila virilis*, I found a certain abnormality in their staining capacities. The small sixth chromosome of the New Orleans strain has this peculiarity; it takes a definitely faint stain than the same chromosomes of other strains.

Two wild American strains of *D. virilis*, New Orleans and New York, sent some years ago by Doctor M. Demerec of the Carnegie Institution of Washington, Cold Spring Harbour, have been inbred in our Laboratory. From their external appearance flies of this type can hardly be distinguished from those of the Japanese wild strains. Detailed comparison shows, however, that the wing length of the former is a little shorter than that of the latter; but such a difference may be found even among wild strains from different localities in Japan—the Keijyo strain, for instance, has the longest wings, Hiroshima the next, and Kyoto the shortest. Both the New Orleans and New York strains have shorter wings than the Kyoto strain. Furthermore the wings of the Kyoto and Hiroshima strains are more pointed at the end of the third longitudinal vein than those of the New Orleans and New York strains. The Keijyo strain has wings as rounded as those of the American. Thus the seeming phenotypical differences between the American and Japanese strains are hardly definite distinctive characters.

The two American wild strains were mated with some Japanese wild strains and the salivary chromosomes of their  $F_1$  larvae were studied. Although nothing peculiar was found in the chromosomes of the hybrid of the New York and Japanese strains, abnormality was found in the sixth chromosome of the hybrid resulting from the cross between the New Orleans and Japanese strains (Figs. 3 and 4).

### Characteristics of the sixth chromosome of the New Orleans *D. virilis*

Maps of the normal salivary gland chromosome have been published by Heitz (1934), Hughes (1936) and this writer (1936).



They agree in that the seventh band from the proximal end is lightly stained, the eighth rather deeply and the ninth lightly, much like the seventh (Bands 100C2-100D3 in Hughes' map and VI1E-VI1G in the writer's). All other bands take a deep stain more or less in the same degree. There is no  $\beta$ -heterochromatin at the proximal end which is attached by its euchromatic region to the  $\alpha$ -heterochromatin. The total length of this chromosome is about 10  $\mu$  (Fig. 1).

As shown in figs. 3 and 4, the sixth chromosome of the hybrid larvae of the New Orleans and Japanese strains consists of two components, one stained as deeply as other chromosomes and the other distinctly lighter. Moreover the component parts are of different lengths, so that by casual observation they appear not to be in synaptic conjugation. Minute studies of the individual bands, however, reveal that the number and arrangement of the bands of the components agree precisely and they are fused band by band. These observations make it certain that the two conjugate completely at somatic synapsis, the abnormality in question thus not belonging to any known chromosomal aberration such as deficiency, inversion or translocation.

Careful comparison of the sixth chromosome of the New Orleans with those of other stocks shows that the New Orleans chromosome is broader and longer, so that the bands, which look continuous in other strains, appear wavy or broken into dots. Thus the chromosome of the New Orleans strain seems to contain the same quantity of chromatic substance as that of other strains.

The sixth salivary chromosomes of 24 strains from various localities of Japan and China, as well as of America, were examined. All of them have normally staining sixth chromosomes except one American stock, Gap<sup>2</sup> hump/lethal 6a, which stained lightly. The original strain of this mutant stock is unknown to us, but it is possibly also New Orleans or else another American strain having a sixth salivary chromosome with the same peculiarity.

#### **Treatment of the New Orleans sixth chromosome with various fixatives**

According to Metz (1935), and Doyle & Metz (1935), preliminary fixation with certain chemicals may give the bands of the salivary chromosomes of *Sciara* a different appearance than when directly stained with aceto-carmine. The salivary chromosome of the New Orleans strain was treated with bichromic acid, formalin, picric acid and mercuric chloride and then stained by aceto-carmine as usual.

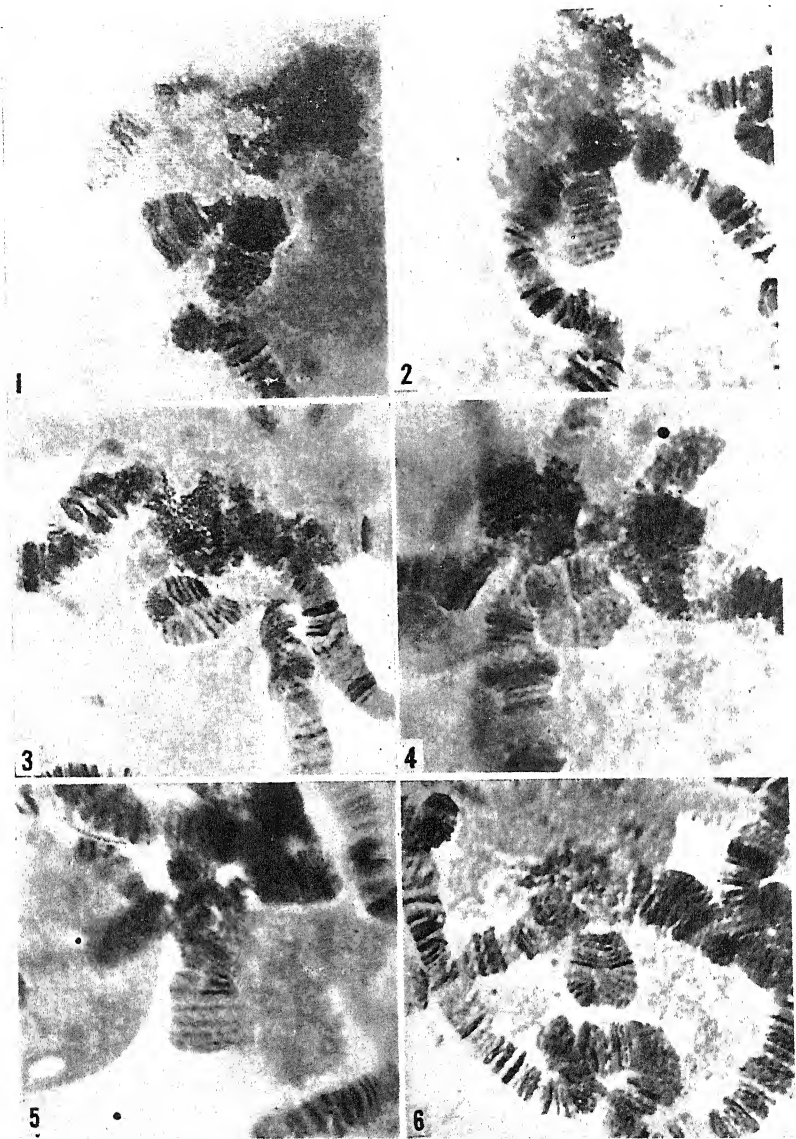


Fig. 1. The normal sixth salivary chromosome in incompleted somatic synapsis. The vacuolar structure, which is attached to the proximal end of the sixth chromosome, is  $\alpha$ -heterochromatin. Fig. 2. The sixth salivary chromosome taken from a fly of the New Orleans stock. This is wider and longer than the normal chromosome. Figs. 3 and 4. The sixth chromosome heterozygous for normal and the New Orleans chromosomes. The difference in staining capacity and length between the two chromosomes can be seen. Fig. 5. The sixth chromosome derived from crossing over between the normal and the New Orleans chromosomes. The left side of the chromosome is the New Orleans and the right side is the new chromosomes whose proximal half is made up of the normal chromosome. Fig. 6. A sixth chromosome of which the left side is the normal and the right side is made up of two kinds of chromosomes—the proximal half of the normal and distal half of the New Orleans chromosome.

*Bichromic acid.* When treated with a solution of 15 grams bichromic acid in 100 cc. of distilled water, the affinity of the chromosome to aceto-carmine is greatly reduced. If the concentration of bichromic acid is reduced to a quarter of the above, the bands become visible but the  $\beta$ -heterochromatin becomes amorphous and hard to observe; the nucleolus is stained clearly, showing a meshwork. The characteristic double appearance of the sixth salivary chromosomes of the hybrids of the New Orleans and other strains is brought out by the differential staining reactions of the components.

*Formalin.* In the salivary chromosomes fixed with fifty percent formalin before staining with aceto-carmine, the distinction between the chromatic and achromatic substances diminishes. The vacuolate nucleolus, however, appears distinctly. Treatment with ten percent formalin does not diminish the distinction between the New Orleans and the normal sixth chromosome.

*Picric acid.* In the material treated with a saturated solution of picric acid diluted with the same volume of distilled water, the salivary chromosomes appear like those in formalin. The New Orleans chromosome can easily be recognized.

*Mixture of formalin and picric acid.* When treated with a mixture of formalin and picric acid, the nucleolus becomes obscure though vacuoles within can be seen. The bands of the salivary chromosomes look similar to those in the material treated with picric acid; the New Orleans chromosome is more lightly stained than the normal chromosome.

*Mercuric chloride.* Mercuric chloride fixation is peculiar in its effect, in that the bands become more granular and the difference between the thick and thin bands becomes more conspicuous in comparison to the case of simple staining with aceto-carmine. The nucleolus becomes obscure and its inner structure invisible.

As stated above, the sixth salivary chromosome of the New Orleans strain appears different, in all the fixatives tried, from the other wild strains. It is thus clear that the abnormal staining capacity of New Orleans chromosome is in no way correlated with any peculiar effect of acetic acid. The chromosomes and their bands can be seen in a living nucleus of the salivary gland mounted in body fluid surrounded by mineral oil. Because of its small size, however, the sixth chromosome can hardly be recognized among the tangled mass of chromosomes. It is therefore not known whether the abnormal staining capacity exists in the living cell or not; but it is likely that even in the living state the density of the chromatin of the New Orleans chromosome is different from those of other strains.

### Crossing over between the normal and abnormal chromosomes

The crossing over in the dot-like sixth chromosome of *D. virilis* was first discovered by Chino and Kikkawa (1933) in their studies of the Japanese stock. They have shown that the crossing over in this chromosome rarely occurs in room temperature, but its frequency can be increased by high temperature, the optimum being 30°C.

Some New Orleans females were mated with males heterozygous for the mutant gene Gap and homozygous for glossy, which belong to the sixth linkage group, and F<sub>1</sub> females were backcrossed with glossy males. The number of F<sub>2</sub> flies and recombination values are summarized in Table 1. The recombination value obtained from

Table 1

Matings	Non-crossovers		Crossovers		Recombination values
	Gp. gl.	+	Gp. or ac. Gp.	gl.	
Hiroshima Gp. gl. × gl. gl.	929	949	1	1	0.11
Keijyo Gp. gl. × gl. gl.	601	619	0	2	0.16
Kyoto Gp. gl. × gl. gl.	904	924	1	0	0.05
Kyoto ac. Gp. gl. × gl. gl.	1007	1042	1	1	0.10
Total	3441	3534	3	4	0.10 ± 0.037
New Orleans Gp. gl. × gl. gl.	657	696	0	0	0
New Orleans Gp. gl. × gl. gl.	931	938	0	1	0.05
New Orleans ac. Gp. gl. × gl. gl.	913	899	1	0	0.06
Total	2501	2533	1	1	0.04 ± 0.028

the female heterozygous for the normal chromosome is  $0.10 \pm 0.037$  and that from the New Orleans chromosome is  $0.04 \pm 0.028$ . The former value is more than twice the latter, but the difference is not statistically significant because of the largeness of the standard error.

The crossover Gap female obtained from the mother, heterozygous for New Orleans chromosomes, died after its emergence. The glossy female was successfully mated with her brother and a stock of this strain was established. This stock has a markedly low fertility

and viability. It is likely that the New Orleans strain gives the same crossover value as the normal strain, but the number of crossovers actually recovered is small on account of their viability. At any rate there is no decisive evidence that the abnormality in the sixth chromosome of the New Orleans stock makes the crossing over in the hybrid strain more difficult than in ordinary cases.

### **Cytological demonstration of crossing over in the salivary chromosome**

The glossy female, which resulted from a crossing over between a normal and a New Orleans chromosome, was mated with a male having the normal sixth chromosome. The  $F_1$  larvae were examined for their salivary chromosomes. About half of the larvae had the normal sixth chromosomes and the other half had chromosomes consisting of a normal and an abnormal haploid (Fig. 6). The abnormal haploid chromosome is made up of a lightly staining New Orleans chromosome in its distal half (VI1A-VI1G) and a deeply staining normal chromosome in its proximal half (VI1F-proximal end). Since the father of these flies was a homozygote for the normal sixth chromosome, it is certain that the abnormal sixth chromosome is derived from the mother which was a crossover between a normal and a New Orleans strain. Thus the genetic result collaborates perfectly with the observation of the salivary chromosomes.

So far cytological demonstrations of crossing over have been made by Creighton & McClintock (1931) and Brink & Cooper (1935) in *Zea mays*, by Stern (1931) with *D. melanogaster* and by Chino & Kikkawa (1933) with *D. virilis*. The observation stated above is another demonstration of this phenomenon which is perhaps even clear than any one by those previous authors.

### **Gap mutant originated from the New Orleans strain**

The mutants so far known in the sixth chromosome of *D. virilis* are acute, abdomen rotatum, Gap, hump, oily, stubby, glossy and lethal 6a (Chino 1936-1937). Of these, Gap was obtained more often than any other mutant and their allelomorphs now amount to ten; they are all dominants and lethal in homo except in certain strains. Another Gap mutant, Gap<sup>11</sup>, appeared in a stock from New Orleans. The salivary chromosome has the expected peculiarity.

### **Further remarks**

The sixth salivary chromosome of the New Orleans strain differs from that in the other strains in its lower staining capacity, probably

on account of the smaller quantity of chromatic material contained. Otherwise it has no peculiarity—either in the arrangement of bands or in the crossingover value, though the data are somewhat inconclusive. Moreover Gap<sup>11</sup>, which is an allelomorph of the Gap mutants, was discovered in the same stock. Only the flies having the chromosomes of the new composition, resulting from crossing over between the New Orleans and normal chromosomes, show markedly lower fertility and viability. This cannot be due to any peculiar genic action of the New Orleans because this stock has normal fertility. Detailed investigations give no evidence of deficiency or duplication of the bands in the chromosome of the new composition. Even if a change had occurred in the achromatic region between the chromatic bands, it is unlikely that such a change would affect the fertility, because the chromatic bands, which represent the loci of the genes, are apparently normal. The last possibility is the position effect of some gene or genes brought into a different chromosome by crossing over. This remains to be shown by more adequate material.

### Summary

1. The sixth salivary gland chromosome of the New Orleans strain of *D. virilis* has a markedly lower staining capacity than that of other strains.

2. This chromosome is also wider and longer than those in other strains. The arrangements of the bands, however, show no difference when crossed, a complete somatic synapsis occurs between this chromosome and the corresponding chromosome of any other strains in the heterozygous condition.

3. Among 23 strains from various localities of Japan and China, as well as of America examined, none had a sixth chromosome like that of the New Orleans. Only a mutant stock, Gap<sup>2</sup> hump/lethal 6a derived from the American Gap<sup>2</sup> stock, contained such a chromosome.

4. No difference was found in the frequency of crossing over between the sixth chromosomes of the New Orleans and that of other strains.

5. In a new composite chromosome derived from such crossing over, the distal half from VI1A to VI1G is made up of the New Orleans chromosome and the other half of a section, VI1F to the proximal end, of a normal chromosome. This gives another case of cytological demonstration of the phenomenon of crossing over.

6. A new allelomorph of Gap<sup>11</sup> was discovered in the New Orleans stock.

7. No significant difference in genetical behaviour exists between the New Orleans and normal strains. Nevertheless the markedly low viability and fertility of the stock with the recombination sixth chromosome is possibly due to some incompatibility between the two kinds of chromosomes.

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## On the Plasmolysis Form in *Allium cepa* with Special Reference to the Influence of Potassium Ion upon It

By

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### Introduction

The results of researches on plasmolysis form and time, started by Weber, Cholodny and others, are now applicable to the examination of the colloidal state of the cytoplasm, especially of its outer layer. It is generally accepted that a convex plasmolysis or a short plasmolysis time indicates a low viscosity of the cytoplasm, and a concave plasmolysis or its long duration means a high viscosity.<sup>1)</sup> But it happens sometimes that contrary to expectation this relation between the viscosity of the cytoplasm and the plasmolysis form appears not to exist, as seen in the case of Cholodny and Sandkewitsch (1933). In the experiments of these authors with epidermis cells of *Allium cepa* it was shown that  $K^+$  and  $Na^+$ , which are generally regarded as decreasing the viscosity of the cytoplasm, act to cause concave plasmolysis, instead of convex, which is found to be caused rather by  $Ca^{++}$ . From these results they assumed that the plasmolysis form is concerned not only with the viscosity of the cytoplasm, but also with some of its other characters. Recently Borries (1938) also remarked in his experiments with *Elodea* that it is hardly possible to examine the normal viscosity of the cytoplasm only according to the plasmolysis form. In the course of the writer's experiments some similar cases to those observed by Cholodny and Sandkewitsch were occasionally met with. This occurred especially when a potassium salt was used as the plasmolyticum. From various points of view it is very important to explain the reason why such an opposite result is obtained, since it has already been mentioned in many monographs and research works, that potassium salts act to cause convex plasmolysis, while calcium salts cause concave. In the present paper a part of the results of the writer's researches on the plasmolysis form and time are reported, in which potassium salt was used as plasmolyticum, which is apt to cause disagreement of the experimental results in many cases.

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1) Weber (1924, a), Küster (1927), Strugger (1935), Heilbrunn (1928).



## Method

1. Material: The inner epidermis of the second scale leaf of *Allium cepa*, was used. The bulbs were those grown in Hokkaido in the previous year. Pieces of the scale about 3–4 quad. mm, were cut off with a sharp razor, and the epidermis was stripped with a pincette from the scale pieces. For each comparative study, the epidermis pieces taken from one and the same scale leaf were used. The osmotic concentration of the incipient plasmolysis corresponded to ca. a 0,55 mol solution of saccharose.

2. KCl solution as plasmolyticum: Glass-redistilled water was used to prepare the solution, and all chemicals were those of Merck or Schering-Kahlbaum. The solutions were prepared in the weight mol.

3. Microscopical preparations: Slide-glass, cover-glass and other glasswares were all those of non-alkaline hard glass. In the study of the plasmolysis form and time,<sup>1)</sup> it is generally necessary to continue the microscopical observation for a long time. In such a case the cell material, which has already lost its turgidity as a result of plasmolysis, may undergo many changes in the nature of the protoplasm due to either the weight of the cover-glass<sup>2)</sup> or the change of the concentration of the plasmolyticum, during the observation. In order to avoid the bad influence of the weight of the cover-glass, capillary tubes, each about 1,5 cm long with both ends closed, were inserted between the slide- and cover-glass. In the case of a long continuing observation the margin of the cover-glass was sealed with vaseline to prevent evaporation of the solution. As the plasmolysis form is apt to be subjected to change on removing the section out of the plasmolyticum, the section was mounted with the plasmolyticum directly on the slide-glass after its removal from the scale leaf.

In the earliest stage of plasmolysis the concave form was generally observed, except for some particular cases. According to the nature of the cytoplasm or to the environmental conditions, the duration of concave plasmolysis, i.e. the plasmolysis time was variable. If however, it may be said that a factor, for instance, a certain ion, causes the convex or concave plasmolysis, this statement is applicable only within a certain limited period. In laps of time the concave form is transformed gradually into the convex one, and finally concave plasmolysis becomes no longer observable. For the study of protoplasmic anatomy it would be most suitable, if the plasmolysis

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1) Weber (1925, a). 2) Sakamura (1933).

time could be exactly measured according to Weber's proposal.<sup>1)</sup> In practice, however, several difficulties are met with in the method of Weber; the microscopical observations ought to be repeated many times, till the convex form appears. But even such repeated observation often does not admit the determination of the plasmolysis time, and the influence of illumination on the plasmolysis, which can not be avoided in this method, is more remarkable than expected, as shown in the following experiments. Therefore, it was rather preferred in the present study, to count the number of the plasmolysis forms within certain periods and to express the tendency of the plasmolysis form in percentage. If in the following explanation the plasmolysis time is not recorded and only the expression "concave" or "convex" is used, it means that one of these forms dominates over the other.

### Explanation of the genesis of different plasmolysis forms

Weber (1924, a) examined the relation between the plasmolysis form and the sling movement of chloroplast in *Spirogyra*, and ascertained that the convex form appears in the material in which the chloroplast can be easily dislocated by centrifugal force, and that in the plasmolysis of such a cell many fine stretched fibres (Hecht's fibres) can not be seen between the cell membrane and the contracted cytoplasm. In the material in which the plasmic contents can be easily dislocated by this force, the concave form appeared and the stretched fibres are produced. From these results he concluded that the convex form indicates a low viscosity of cytoplasm and the concave form a high viscosity. According to the same author (1925, b), however, the plasmolysis form should depend upon the following factors at least: 1. Viscosity of the endoplasm. 2. Adhesion of the cytoplasm to the cell membrane. 3. Consistency of the chloroplast. Considering these points Weber assumed that convex plasmolysis means a very weak adhesion of cytoplasm to the cell membrane and a rather liquefied state of the endoplasm, i.e. its low viscosity, while a concave plasmolysis occurs in the cytoplasm of high viscosity. Even if, contrary to the general expectation, a case is met with where potassium salt causes concave plasmolysis and calcium salt convex,<sup>2)</sup> it would not be necessary hastily to accept a different explanation that  $K^+$ ,  $Na^+$  and  $NH_4^+$  increase the viscosity of the protoplasm and  $Ca^{++}$  decreases it.<sup>3)</sup> In order to explain such inconsistent results it is necessary to take into consideration not only the viscosity

1) Strugger (1935).

2) Cholodny und Sandkewitsch (1933). 3) Heilbrum (1928. p. 147).

of protoplasm, but also other related factors, without abandoning the general idea that  $K^+$  decreases the viscosity of the protoplasm and  $Ca^{++}$  increases it. For the purpose of this explanation it is not enough to speak about the viscosity of the whole protoplasm, but the adhesion of the cytoplasm to the cell membrane must also be considered.<sup>1)</sup>

When the cytoplasm contracts in the plasmolysis, it separates from the cell membrane easily or with difficulty according to the degree of the adhesion between the cytoplasm and cell membrane. As also the cohesion of the cytoplasm, which has much to do with its viscosity, ought to participate in its contraction, it is natural to assume that competition occurs between the adhesion and cohesion, if the cytoplasm does not easily separate from the membrane. From these reasons it is important to consider that the plasmolysis form may depend upon this relation between the adhesion and cohesion. The factor which increases the adhesion is apt to cause concave plasmolysis and that which decreases it tends to cause convex plasmolysis in general.

If a certain plasmolyticum has a special effect upon the colloidal state of the cytoplasm, the proper relation between the adhesion and cohesion will be modified by it and the other plasmolysis form may appear according to the new condition. As the result of such action of the plasmolyticum, especially of electrolyte, mention can be made of the condensation resp. loosening of the micellar structure at the outer layer of the cytoplasm and also the decrease of the cohesion or viscosity of its inner part owing to the penetration of the plasmolyticum (intrability!). If some physical factors come to act in addition to this, the proper rival relation between the adhesion and cohesion will be more complicated. Generally it may be considered probable that a relatively strong adhesion or a relatively weak cohesion tends to cause concave plasmolysis, and in the latter case it is often abnormal.

Mostly depending upon his own microscopical observation the writer wants to attempt an explanation of the genesis of the different plasmolysis forms. If the cytoplasm separates with relatively great difficulty from the cell membrane, a normal concave plasmolysis results. If the plasmolyticum quickly penetrates into the cytoplasm, the cohesion becomes weaker than the adhesion and the inner part of the cytoplasm more or less tends to plasmoschisis before the plasmolytic contraction begins. In such a case concave plasmolysis happens, which is, however, of another kind and sometimes of abnormal

1) Weber (1925, b).

nature<sup>1)</sup> and of which the extreme form is called tonoplast plasmolysis. When the adhesion is strong in both these cases of concave plasmolysis, the cytoplasm remains attached partially to the cell membrane, and the wall pattern and stretched fibres of it are produced. The substance which forms these figures perhaps originates from the periphery of the cytoplasm, but it is possible that the endoplasm also participates often in these phenomena, if its cohesion is decreased.

Besides concave and convex plasmolysis, cap plasmolysis occurs not seldom. According to the view of Höfler (1934) the cap plasmolysis would appear when convex plasmolysis is formed in the beginning and then the cytoplasm gradually swells at both its narrow ends, requiring a pretty long time for its complete formation. However, the writer's observations indicate that cap plasmolysis can be

caused sometimes by other geneses, besides that of Höfler, within a relatively short time.

In the following (Figs. 1 & 2) the writer wants to classify the plasmolysis form in the epidermis cell of *Allium cepa* according to the process of its formation, while the results of his studies on this problem will be reported in detail in future.

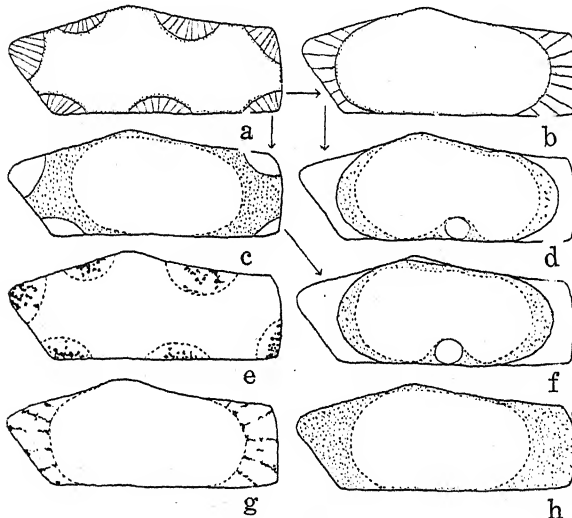


Fig. 1. a, schema 1; b, schema 2; c, schema 3; d, schema 4; e, schema 5; f, schema 6; g, schema 7; h, schema 8.

Schema 1. Normal concave plasmolysis. Stretched fibres and wall pattern are observed.

Schema 2. Convex plasmolysis. Plasmolysis begins in the concave form. The outer layer of cytoplasm, which partially remains attached to the cell membrane in the beginning, separates gradually, and the plasmolysis takes the convex form at last.

1) Küster (1929, p. 30, Fig. 7).

Schema 3. Cap plasmolysis. In some cases the convex plasmolysis shown in Schema 2 is transformed to a cap plasmolysis of Höfler. This form will be regarded as the result of swelling of both ends of the cytoplasmic body toward the outside.

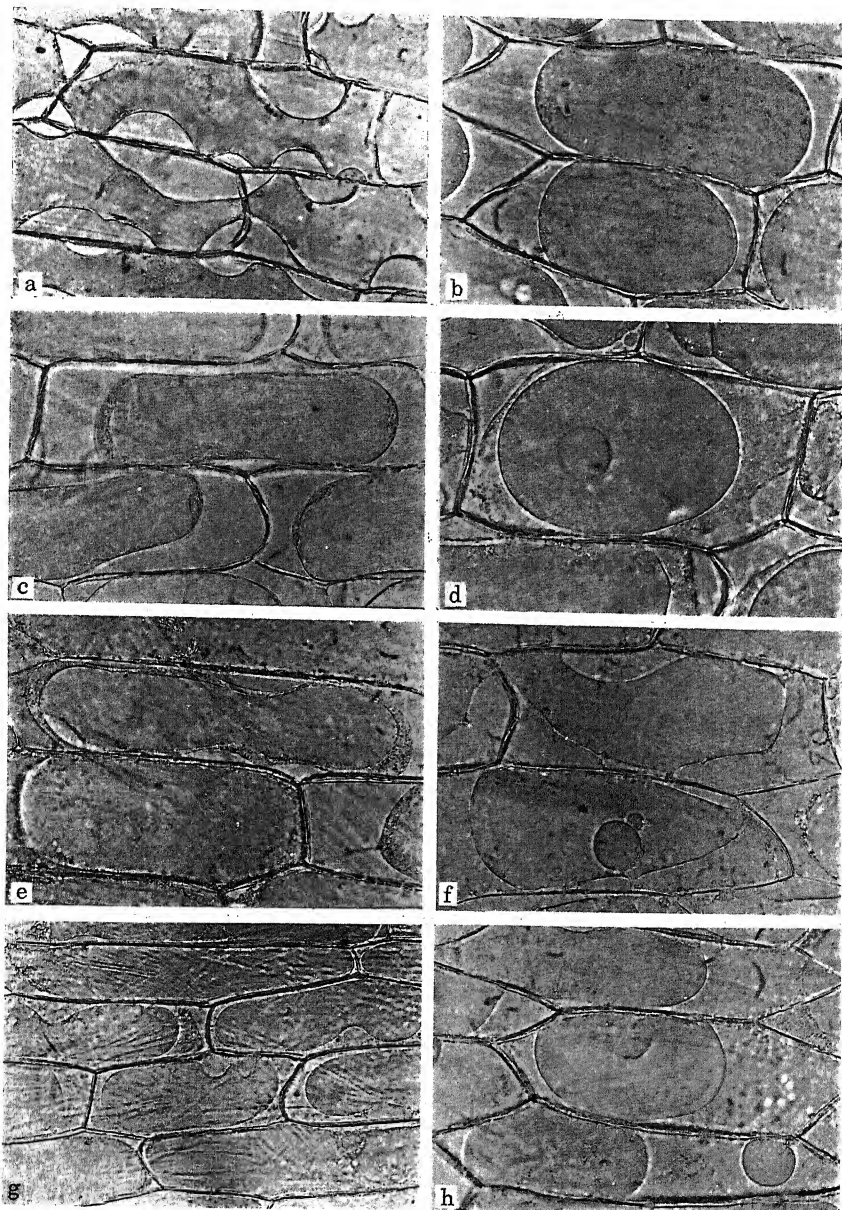


Fig. 2. a, schema 1; b, schema 2; c, schema 3; d, schema 4; e, schema 5; f, schema 6; g, schema 7; h, schema 8.

Tonoplast remains without elastic movement. Swelled hyaline nucleus gets out of cytoplasm.

Schema 4. Tonoplast plasmolysis. Cohesion of the endoplasm in the concave plasmolysis of Schema 1 decreases and the tonoplast contracts toward the inside. The outer layer of cytoplasm partially remains resting on the cell membrane. This plasmolysis form is an example showing the relation cohesion < adhesion.

Schema 5. Cap plasmolysis. The attached parts of the outer layer of cytoplasm in Schema 4 gradually separate from the cell membrane and the surface becomes smooth-convex. This is apparently the same as the tonoplast plasmolysis of Schema 3. Hyaline nucleus gets out of cytoplasm.

Schema 6. Abnormal concave plasmolysis. In the beginning an abnormal concave plasmolysis occurs, which is very similar to the tonoplast plasmolysis. Its difference from the normal concave plasmolysis is that the large part of cytoplasm remains attached to the cell membrane or is scattered in suspended state in the interspace between the contracted part of the cytoplasm and the cell membrane. The concave surface is very thin and seems to consist mostly of tonoplast alone.

Schema 7. Tonoplast plasmolysis. This is derived from Schema 6. Cytoplasm is scattered in suspended state in the interspace.

Schema 8. Tonoplast plasmolysis. The appearance is similar to Schema 7. This is performed by sudden contraction of tonoplast alone in the beginning. Cytoplasm remains attached to the cell membrane or scattered in the interspace. Pretty thick stretched fibres appear between the cytoplasm and cell membrane.

It is generally known that, when KCl is used as a plasmolyticum, convex plasmolysis is liable to occur, but it is necessary to examine whether this plasmolysis form is always really normal convex. Such an examination can be done by adequate observation with a satisfactory illumination and magnification. Without this precaution it happens sometimes that a tonoplast plasmolysis is taken for a real convex plasmolysis. The contracted cytoplasm in the normal convex plasmolysis has a certain remarkable thickness and refracts light more or less intensively, while in the tonoplast plasmolysis the surface is very thin and vague and granular substances of the cytoplasm are scattered in the interspace.

## The relation between the plasmolysis form and various factors

### 1. Saccharose solution

When a KCl solution, of which  $K^+$  has special influence upon the plasmolysis form, is used as the plasmolyticum, it is necessary to make some observations of plasmolysis in non-electrolyte solution, such as saccharose solution, in comparison.

a) 0,6 mol solution. The separation of the cytoplasm from the cell membrane began gradually at the corner of the cell and a concave form appeared (Fig. 3).

Streaming of the protoplasm, granular suspension and no vacuolisation of the cytoplasm were visible similarly to the normal state of the non-plasmolysed cell, which could not be the case in the KCl-plasmolysis. The inner surface of the contracted cytoplasmic layer was kept smooth for a long time, without any localized accumulation of cytoplasm there. After a certain period the plasmolysis turned into the convex form, but neither cap nor tonoplast plasmolysis was found.

b) 1 mol solution. The process of plasmolysis was nearly the same as in the case of a 0,6 mol solution, except that the plasmolysis began in the concave form somewhat slowly and the plasmolysis time lasted longer. Derry (1930) also ascertained the same difference of the plasmolysis time in *Spirogyra* between the saccharose solutions of various concentrations.

Figs. 3 and 4 show the progress of the plasmolysis caused by a 0,6 mol and 1 mol saccharose solution respectively. The progress

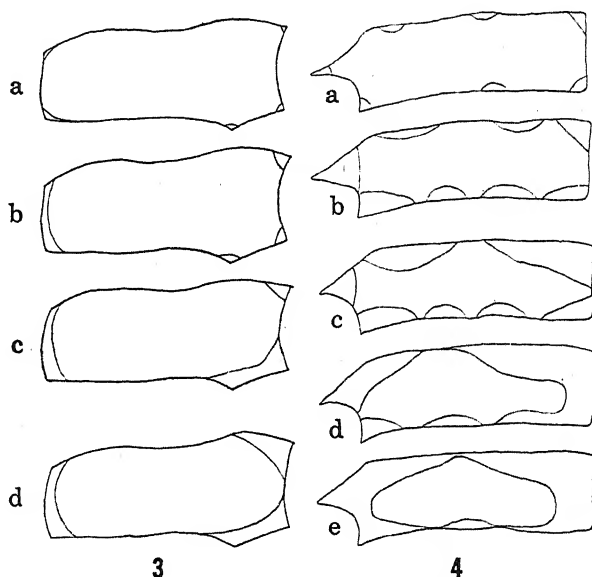


Fig. 3. 0,6 mol saccharose solution. a, after 1 minute; b, after 5 minutes; c, after 15 minutes; d, after 30 minutes. Fig. 4. 1 mol saccharose solution. a, after 1 minute; b, after 5 minutes; c, after 15 minutes; d, after 45 minutes; e, after 1 hour 15 minutes.

was followed using an Abbe's drawing apparatus. The plasmolysis time was 30 minutes in the 0,6 mol solutions and 1 hour 15 minutes in the 1 mol solution. By a very careful microscopical observation with strong illumination only a few stretched fibres were visible, but the wall patterns never came in sight. It seems likely that the loss of water from the vacuole during the plasmolysis accompanies the dehydration of the cytoplasm, which probably increases the adhesion between the cytoplasm and cell membrane as well as the cohesion of the cytoplasm itself. As it is scarcely doubted that such increase of the adhesion in the hypertonic sugar solution is not so strong as in the  $\text{CaCl}_2$  solution, the concave form, with which the plasmolysis begins, can be transformed into the convex form more easily in the former solution than in the latter. If a sugar solution of extremely high concentration is, however, used as the plasmolyticum, this tendency of the change of the plasmolysis form will be naturally delayed. The effect of the hypertonic sugar solution on the plasmolysis form is at any rate different from that of electrolyte in the fact that the pure osmotic phenomenon alone is concerned in it.

## 2. KCl solution

Though it is generally believed that the plasmolysis time in a hypertonic KCl solution is very short, the writer has experienced that this is not always the case, but that the time is variable according to the material and the condition of preservation and observation. For instance, the concave form continued three hours or longer in one extreme case, when epidermis cells were plasmolysed with a 0,5 mol KCl solution, while in most cases it turned into the convex form within 1-2 hours. During the transition from the concave to the convex plasmolysis, in a 0,5 mol KCl solution, the stretched fibres and wall pattern appeared (Fig. 5), the formation of which might be participated in by the ectoplasm and sometimes also by a part of the endoplasm. Besides the transformation from the concave into the normal convex plasmolysis (Schema 2), the cap plasmolysis (Schema 3) occurred or the concave plasmolysis turned at last into the tonoplast plasmolysis in abnormal cases (Schema 4, 8, Fig. 2, h, g and Fig. 6).

In a 1 mol KCl solution, the contracted cytoplasm swelled up or the concave tonoplast plasmolysis began to appear, soon after the beginning of the plasmolysis.

When the material, which had been kept in a 0,025 mol KCl solution for 20 hours, was plasmolysed in a 0,75 mol saccharose solution, the plasmolysis time was very short. The same thing happened also in the plasmolysis caused with a  $3/4$  mol saccharose solution after



treatment in a 0,16 mol KCl solution, and the tonoplast plasmolysis was brought about.

From the results of the observation described above, it may be said that the plasmolysis form or time is very variable according to the nature of the cytoplasm or to the experimental conditions, even when exactly like materials are used. Such relations have been studied by Derry (1930), and it was also confirmed by Weber (1925a), Satō (1925), Strugger (1935) and others that the plasmolysis form largely depends upon the stage of development even in the same organ.

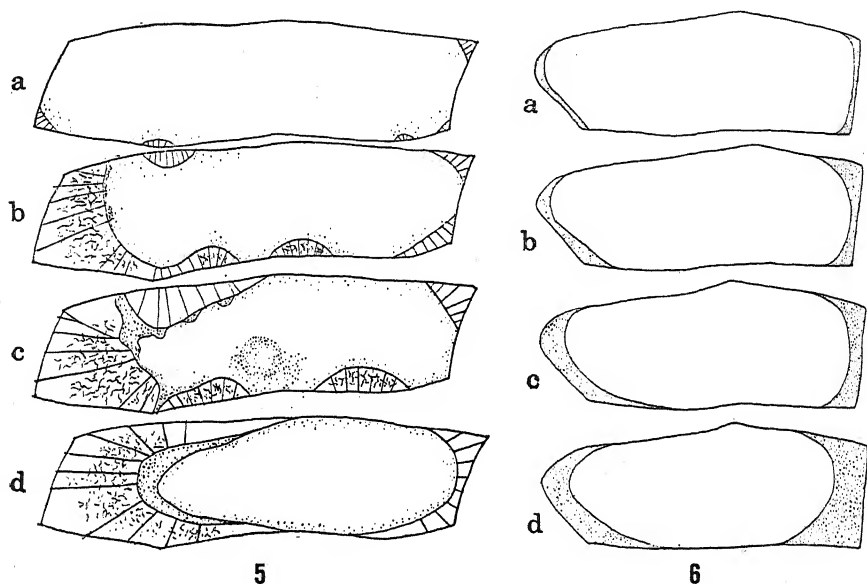


Fig. 5. 0,5 mol KCl solution. a, after 1 minute; b, after 10 minutes; c, after 30 minutes; d, after 1 hour. Fig. 6. 0,5 mol KCl solution. a, after 1 minute; b, after 5 minutes; c, after 15 minutes; d, after 30 minutes.

Some results of the present writer's experiments show that the difference of the plasmolysis forms which appears according to the nature of the cytoplasm or to the experimental condition, is more remarkable in KCl solution than in sugar solution. Accordingly it is advantageous to use KCl solution as plasmolyticum, when the nature of the cytoplasm is examined by means of the plasmolysis form, though the extreme liquefying effect of this salt on the cytoplasm often declines with the result that it is difficult to reach a conclusion. Such difficulty may be avoided by the investigation of the factors which cause variation of the plasmic nature. To clear up these relations is the principal aim of the present work.

### 3. Material

In order to examine the difference of materials as influenced by the temperature, they were plasmolysed with hypertonic saccharose solution, because it does not show any special action besides the plasmolytic dehydration. The bulbs had been conserved in the cellar of the market and appeared very fresh. The experiments were conducted in the middle of summer in Sapporo. The plasmolyticum was 1 mol saccharose solution.

a). Material kept one day in an ice box ( $10^{\circ}\text{C}$ ). Plasmolysis hardly occurred in most cases 30 minutes after the transference of material into the plasmolyticum.

b). Material kept one day in a laboratory room ( $25^{\circ}\text{C}$ ). In most cases concave plasmolysis was seen 30 minutes after the transference.

c). Material kept 2 days in an ice box ( $10^{\circ}\text{C}$ ). Concave plasmolysis after 15 minutes.

d). Material kept 2 days in a laboratory room ( $25^{\circ}\text{C}$ ). Tonoconvex plasmolysis after a few minutes.

The adhesion between the cytoplasm and cell membrane may be intensified by the osmotical dehydrating action of a hypertonic saccharose solution; the plasmolysis begins ordinarily in the remarkable concave form and it continues for a while. From the above experiment it may be recognized, that the lower the temperature of preservation is, the more remarkable this tendency appears. If the material is kept at high temperature, the adhesion seems, on the contrary, to be weakened and sometimes tonoplast plasmolysis happens, which may indicate even the intrability of the cytoplasm for saccharose. It is now clear, that even when saccharose solution, which does not act so intensively on the colloidal state of the cytoplasm as any electrolyte, is used as plasmolyticum, the influence of temperature during the preservation appears very noticeably. This fact naturally suggests that such influence of temperature would be intensified by the use of KCl as the plasmolyticum.

### 4. $C_H$ of KCl solution used as plasmolyticum

In connection with the influence of  $C_H$  on the plasmolysis time Derry (1930) has proved that the plasmolysis time in *Spiragyr*a cells, which were plasmolysed with a 25% saccharose solution, was long in the large pH values and short in the small pH values.

In the present case the pH value of 0,5 mol KCl solution was variated with diluted KCl or  $\text{KHCO}_3$  solution and the plasmolysis form was observed after 30 minutes.

Except pH 7,0 concave plasmolysis appeared at first.

pH 7,0 Immediately tonoplast-convex plasmolysis in most cells.

Pretty large cytoplasmic granules remain in the interspace.

pH 6,6 Tonoplast plasmolysis.

pH 5,6 Concave plasmolysis, in which also a tendency to tonoplast plasmolysis is visible.

pH 5,3 ditto, and granules of cytoplasmic remain scattered in the interspace.

pH 4,6 ditto.

pH 4,0 Concave plasmolysis, which tended finally to tonoplast plasmolysis. Localized cytoplasmic remains on the cell membrane. adhesion > cohesion (Schema 6 or 8).

pH < 4,0 Concave plasmolysis continued longer than in pH 4,0. The adhesion was much more intensified.

From the above experiment it will be seen that the liquefying effect of  $K^+$  on the cytoplasm increases in high pH values, especially in pH 7,0. In such condition  $K^+$  penetrated into the cytoplasm rapidly and caused the diminution of the cohesion of its inner part, which seemed to precede the separation of the cytoplasm from the cell membrane. As the result of this process tonoplast plasmolysis appeared in many cases, instead of convex plasmolysis. In small pH values this tendency was reduced to some degree, but it could not be completely eliminated. The result of Derry's work, in which saccharose as plasmolyticum was used, can not be immediately consulted here, because in the writer's case the action of  $K^+$ , especially in high pH value, comes additionally into consideration.

## 5. Light

Weber (1929d) plasmolysed the cell of the spongy tissue of the "Sonnenblatt" and "Dunkelblatt" of *Ranunculus ficaria* with KCl and  $CaCl_2$  solution, and found concave plasmolysis and long plasmolysis time in the former and convex plasmolysis and short plasmolysis time in the latter. Whether this result was caused directly by light or secondarily induced, he did not say distinctly. Huber (1926) confirmed a similar relation between the sun leaf and shade leaf of *Elodea*.

In his own experiment the writer happened to see that in the epidermis cell of *Allium cepa*, plasmolysed with a 0,5 mol KCl solution, the plasmolysis time was remarkably shortened and after one hour tonoplast or cap plasmolysis appeared, when the microscopical observation was often repeated. The microscope was provided with a light condensor, and the diffused sun light or a Leitz low-voltage lamp "Monla" was used as the light source, applying Zetnow's colored

solution as the light filter. The plasmolysed cell kept in the dark space, however, continuously showed concave plasmolysis after the same duration. Probably temperature exerted no influence, because the temperature on the microscopical stage and in the dark place was equally 25°C.

In order to ascertain such direct influence of light on the plasmolysis form a further experiment with the apparatus illustrated in Fig. 7 was carried out.

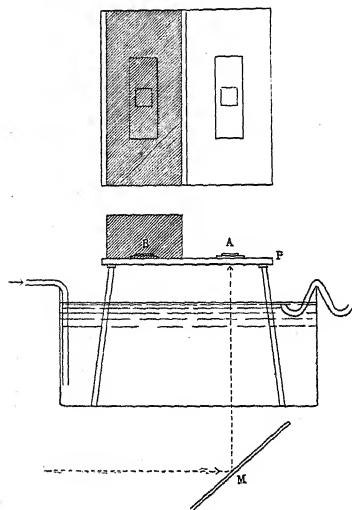


Fig. 7. The preparation in part A was illuminated from below by projected light, and exposed to diffused light from above. In part B the projected light from below was intercepted with a sheet of black paper and the preparation was covered with a cardboard case. The preparation was, therefore, kept completely dark in that part. P, Glass plate; M, Mirror.

One hour after the beginning of the experiment different results caused by the illumination and obscuration were clearly confirmed. The former remarkably accelerated the transformation from concave to convex plasmolysis (Figs. 8, 9).

In another experiment a part of the preparations was kept on the table by the window, being exposed to diffused sun light, and the other part was placed in an obscure position (Figs. 10, 11). The temperature was equally 25°C. After one hour the amount of remaining concave plasmolysis was determined in percentage (Table 1).

Table 1

	Material				
	1	2	3	4	5
Illuminated	12,2	10,7	13,1	12,4	25,4
Dark	41,5	37,8	98,0	97,9	88,5

From this result it is clearly seen that the light has a non-negligible influence upon the plasmolysis form. By many authors it has been proved that the light increases the permeability of the protoplasm. This fact makes it possible that the light causes also colloidal change which is represented by the decrease of the adhesion between the cytoplasm and cell membrane as well as of the cohesion of the cytoplasm itself. Such influence of the light could not, however, be seen when  $\text{CaCl}_2$  was used, as shown in the following experiment (Table 2). In the table the occurrence of concave plasmolysis is given in percentage after one hour.

The above described fact shows that if one wishes to know the nature of the protoplasm judging from the plasmolysis form or time, it is necessary to select the plasmolyticum and to conduct the ob-

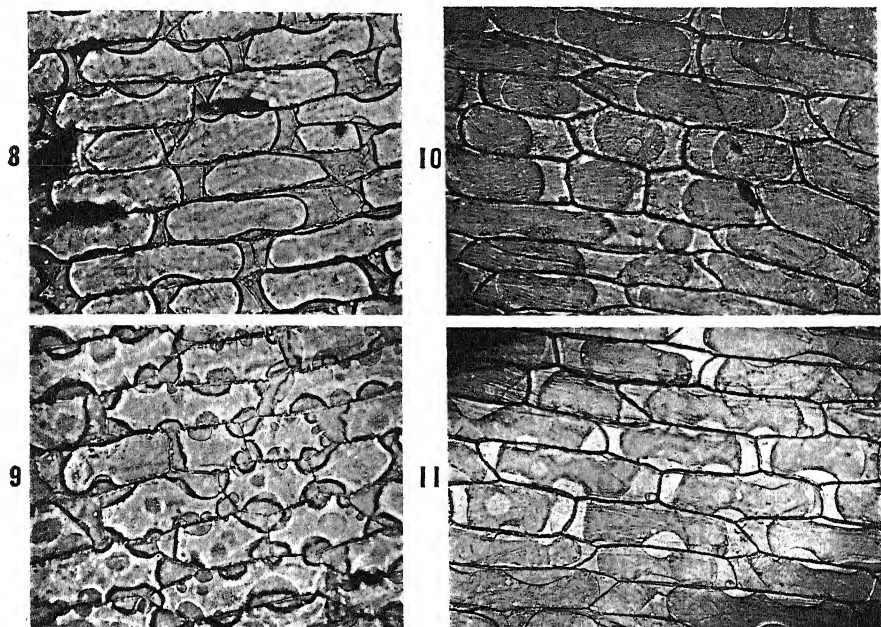


Fig. 8. Illuminated. Fig. 9. Dark. Fig. 10. Illuminated. Fig. 11. Dark.

servation under proper conditions of light. Especially in the case of delicate material, it is necessary to avoid the repeated microscopical observation using a strong illumination, because it may very probably modify the results.

Table 2

	Material	
	1	2
Illustrated	87,1	84
Dark	87,3	83,6

## 6. Temperature

Weber (1932b) found that the plasmolysis permeability to urea in the cell of leaf epidermis of *Elodea canadensis* is increased by cooling the material at 1°–3°C. He attributed this to the mechanical ill effect which is caused by the forced separation of the cytoplasm from the cell membrane in the face of the raised adhesion between them and to the failure of recovery of the normal surface of the cytoplasm at low temperature. Derry (1930) found in *Spirogyra* that the plasmolysis time was infinitely long at 0°C, and it became shorter in proportion with increase in temperature, showing again an increasing tendency above 25°C. Concerning the influence of tem-

perature upon the normal permeability which has much to do with the colloidal state of the cytoplasm, it is generally believed that it increases at relatively high temperature. In the previous experiment it was clearly shown that the temperature during the preservation remarkably affects the plasmolysis form. From these facts it may be expected that when KCl is applied as the plasmolyticum the temperature also somewhat influences the plasmolysis form during the experiment.

An Eisenberg's heating and cooling stage was used, which permits the maintenance of a constant temperature of the preparation. A square glass frame of 3 mm high was stuck with Canada balsam on a slide-glass; this served as a pool which was filled with the plasmolysing solution. The section of epidermis was immersed in the solution, then the pool was covered with a cover-glass and kept on the stage dark. At the time of the microscopical observation the cover-glass was taken off, because dew-drops, which were formed on the lower surface of the cover-glass at the low temperature, hindered the observation.

In a 0,5 mol KCl solution the epidermis cell showed the concave plasmolysis at first and after one hour the number of the remaining concave form<sup>1)</sup> was counted. The rate is given in percentage (Tables 3 & 4).

Table 3

Stage temperature	After 30 minutes	After one hour
15°	98,8	95,2
20°	9,1	86,3
25°	91,2	53,7
30°	68,9	42,8

Table 4

Stage temperature	After 30 minutes	After one hour
20°	74,1	57,9
25°	37,4	11,4
30°	20,6	8,4
35°	4,9	1,0

The experiment on this line was repeated several times, and the similar result was obtained every time, except that sometimes a reverse relation of percentage was found between 20° and 25°C. At higher temperature than 25°C the tonoplast and cap plasmolysis were observed. The plasmolysis tendency to the convex form or often to the tonoplast and cap plasmolysis at high temperature may be explained by the decrease of the adhesion between the cytoplasm and cell membrane, and by the increase of the intrability, similarly to the case of the influence of high temperature during the preservation as shown in the previous experiment.

1) The convex, tonoplast and cap plasmolysis may be mentioned as the forms other than the concave. When tonoplast and cap plasmolysis appear frequently, concave plasmolysis is rare.

### 7. Humidity during the preservation of material

Here, the preservation of material means that done during the time previous to the experiment. In order to obtain dry or moist condition some desiccators were used, either containing calcium chloride or without it. In the latter case the air within the desiccator was saturated with water vapor coming from liquid water at the bottom and from wet filter paper on the side wall. The plasmolysing solution was a 0,5 mol KCl solution. Temperature: 24°C. Preservation time: 24 hours. The observation was made 30 minutes after the immersion of the material. The number of the remaining concave form is given in percentage (Table 5).

In another experiment bulbs were preserved in an ice-box, either in dry or wet condition. The temperature was kept at 10°C. The observation after 15 minutes showed that in the dry condition the plasmolysis was mostly convex and in the wet it continued in the concave form. This experiment was repeated with the same results. Here, a general tendency is recognizable that in the wet condition the normal concave plasmolysis can be kept for a pretty long time without change, and that in the dry condition the material hurries to take the convex form, sometimes showing the tonoplast plasmolysis which can be, however, prevented from taking place by keeping the material cool.

Table 5

	Bulb			
	1	2	3	4
Dry	33,4	70	76	23,6
Wet	98,0	92	95	91,5

### 8. Immersion of material in water

The foregoing experiments show that the plasmolysis time can be much prolonged by keeping the material in wet condition. Therefore, it is not unnecessary to see, whether the immersion of material in water may give the same result.

A scale leaf was cut in halves and one half was left on the table at 25°C without cover and the other was immersed in tap water of two kinds, still standing water (25°C) and running cool water (11°C), for 30 minutes. Epidermis cells were plasmolysed with a 0,5 mol KCl solution and microscopical observation was made after 30 minutes (Table 6).

From this result it may be said that the preservation of the scale leaf in cool water is very effective to reduce the ill tendency of the cytoplasm which is seen when it is kept in the atmosphere at high temperature, while the immersion of the material in water of high

temperature is useless for the purpose of reducing that ill tendency. It is a very remarkable fact that even the cell, which is in an abnormal condition and which shows such an ill tendency as tonoplast plasmolysis, can easily be restored to normal condition by the cool immersion. With the striped epidermis a similar result was obtained.

Table 6

	Material		
	1	2	3
In the atmosphere (25°C) Immersed (25°C)	tonopl., cap tonopl., cap	tonopl., concave tonopl., concave	tonopl., cap tonopl., cap
In the atmosphere (25°C) Immersed (11°C)	tonopl. concave	tonopl. concave	tonopl. concave

In the study on the plasmolysis form and time with epidermis cell of *Allium cepa* it happens sometimes that the materials, even of the same origin, yield different results according to the condition during preservation. In one case the concave plasmolysis continues very long, while in another case it is soon transformed into the convex form, which has a great tendency towards the tonoplast or cap plasmolysis. The material in the latter case can never be regarded as thoroughly healthy. Therefore, it is necessary for the purpose of the study of the plasmolysis form to find a suitable condition under which the material can be preserved against showing such an ill tendency of the plasmolysis form.

From the results of the above mentioned and of additional experiments,<sup>1)</sup> the several conditions for the preservation may be summarized as follows:

1. Immersion in cool tap water (11°C) . . . . . best (Fig. 12)
2. Moist cool chamber (10°C) . . . . . good
3. Cool chamber (10°C) . . . . . pretty good
4. Moist chamber (25°C) . . . . . pretty good
5. Laboratory room (24°-25°C) . . . . . bad
6. Dry chamber (24°-25°C) . . . . . bad
7. Warm dry chamber (30°C) . . . . . bad (Fig. 13)

#### 9. Antagonistic action of Ca<sup>++</sup> against K<sup>+</sup>

It has been confirmed by many authors that the plasmolysis forms caused by a Ca-salt are different from those caused by a K-salt.

1) Additional experiments were carried on with several combinations of factors. The protocols of these experiments are omitted here, because they are no more than confirming the writer's previous experiments.



In order to compare the results of the above experiments, where KCl was used as plasmolyticum, with the effects on the plasmolysis form of  $\text{CaCl}_2$  and to ascertain its antagonistic action against KCl, the following experiments were conducted.

a) 0,25 mol  $\text{CaCl}_2$  solution. The plasmolysis went on very slowly and the separation occurred only at the corners, the concave form resulting in an inconspicuous manner (Fig. 14).

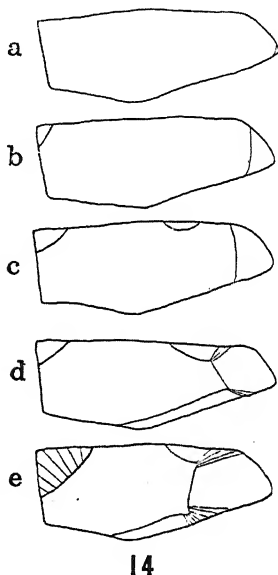
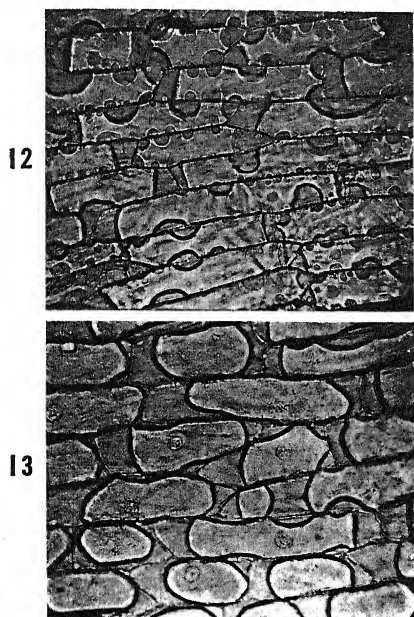


Fig. 12. The bulb of *Allium cepa* immersed 24 hours in the cool tap water (11°C).  
 Fig. 13. The bulb of *Allium cepa* is kept 24 hours in the thermostat (30°C).  
 Fig. 14. 0,25 mol  $\text{CaCl}_2$  solution. a, after 1 minute; b, after 15 minutes; c, after 30 minutes; d, after 1 hour; e, after 2 hours.

b) 0,3 and 0,5 mol  $\text{CaCl}_2$  solution. The plasmolysis began immediately in the concave form, and the stretched fibres and wall pattern were clearly visible. This concave plasmolysis continued for a pretty long time, sometimes longer than 2 hours and in an extreme case 5 hours. This may be explained by an assumption that  $\text{Ca}^{++}$  increases the adhesion between the cell membrane and cytoplasm, and this cation itself barely penetrates into the inner part of the cytoplasm; it has, therefore, no influence upon the cohesion or viscosity in the inner part of the cytoplasm. In the KCl-plasmolysis as well as the  $\text{CaCl}_2$ -plasmolysis it begins in the concave form, but in the former it continues for a shorter time and is more easily transformed into the tonoplast or cap plasmolysis than in the latter. This is most-

ly due to the difference in the process of the plasmolysis between the two cases, as previously stated.

As  $\text{Ca}^{++}$  acts antagonistically in various relations, especially on the colloidal state of the protoplasm, against  $\text{K}^+$ , it is reasonable to expect that the characteristic feature of the plasmolysis caused by KCl-solution is modified by an addition of a small amount of  $\text{CaCl}_2$ . Plasmolytica of the following three kinds were used in the experiment:

1. 0,5 mol KCl.
2. 0,3 mol  $\text{CaCl}_2$ .
3. 0,5 mol KCl (95 parts) + 0,3 mol  $\text{CaCl}_2$  (5 parts).

The difference of the osmotic value among these solutions may be neglected, because it is so slight that it cannot cause any variation of either the plasmolysis form or time. Temperature of the plasmolytica:  $23^\circ\text{C}$ . In the following tables the occurrence of the concave form after 30 minutes is given in percentage (Table 7).

In another experiment the concentration of  $\text{CaCl}_2$  was increased to 0,4 mol (Table 8).

Table 7

	Material		
	1	2	3
KCl	32,2	50,8	36,9
$\text{CaCl}_2$	100	100	100
$\text{KCl} + \text{CaCl}_2$	61,6	92,1	76,7

Table 8

	Material		
	1	2	3
KCl	84,3	45,5	45,8
$\text{CaCl}_2$	100	100	100
$\text{KCl} + \text{CaCl}_2$	94,9	94,6	97,3

$\text{Ca}^{++}$  acts clearly against  $\text{K}^+$  and prolongs the plasmolysis time remarkably. Similarly to other protoplasmic antagonism this may be interpreted firstly that  $\text{Ca}^{++}$  increases the adhesion between the cell membrane and cytoplasm, and secondly that it restricts the entrance of  $\text{K}^+$ , which will give rise to the abnormal plasmolysis.

#### 10. Antagonistic action of $\text{Al}^{+++}$ against $\text{K}^+$

From the antagonism between  $\text{Ca}^{++}$  and  $\text{K}^+$  in relation to the plasmolysis form, it may not be unreasonable to expect a similar result, when  $\text{Al}^{+++}$  is used instead of  $\text{Ca}^{++}$  as the antagonist, because  $\text{Al}^{+++}$  dehydrates the surface of the cytoplasm more effectively than  $\text{Ca}^{++}$  (Weber 1924b). The following solutions were used in the present experiment.

1. 0,5 KCl (pH 5,8).
2. 0,001 mol  $\text{AlCl}_3$  in 0,5 mol KCl (pH 4,2).
3.  $\text{AlCl}_3$  in various plasmolysing concentrations.

As the pH-values of two plasmolytica 1 and 2 used in the above experiment were not the same, that of solution 1 was regulated to 4.2 with HCl (Table 10).

Table 9

Plasmolyticum	Plasmolysis form	
	After 15 min.	After 30 min.
1	convex, tonopl.	convex, tonopl.
2	concave	concave
3	cramp	cramp

Table 10

Plasmolyticum	Plasmolysis form	
	At once	After 30 min.
1	concave	tonopl.- concave
2	concave	concave

It is a note-worthy fact that  $Al^{+++}$  which is commonly regarded as an outsider for keeping the protoplasmic condition normal, acts very effectively against  $K^+$ , suppressing the tendency towards tonoplast plasmolysis.

#### 11. Reexamination of the result of Cholodny and Sandkewitsch

In some experiments of Cholodny and Sandkewitsch (1933) epidermis cell of *Allium cepa* were previously immersed in hypotonic solutions of  $NH_4^+$ , Na-, K-, Mg- and Ca-chloride, and after a certain period the cells were plasmolysed with a 3/4 mol saccharose solution. All of the alkali salts caused the convex or transverse plasmolysis and the alkaline earth salts the concave plasmolysis. However, when the materials were previously treated with isotonic solutions of these salts reverse results were obtained, that is, the alkali salts caused concave, and the alkaline earth salts convex plasmolysis. From this result they concluded that the form and process of the plasmolysis depend not only on the viscosity of the protoplasm, but also on its other unknown characters. If their experimental result is sufficient basis for this conclusion, one must very carefully apply the plasmolysis form method for the purpose of the investigation of the ion action on the protoplasmic character.

In the present work the experiment of Cholodny and Sandkewitsch was reexamined with the same procedure. The results obtained are given in the following table with their results for comparison (Table 11).

The results of A 1, A 2 and B 2 were in accord with those of Cholodny and Sandkewitsch, but in the case of B 1 the writer's experiment resulted entirely differently and shows no tendency inconsistent with the result in A. In the material of B 2, previously treated with  $CaCl_2$ , the plasmolysis form observed after 3 hours is convex,

but it might be concave, if the observation were made after 25–35 minutes as in A. As, in B 1 of the writer's experiment, convex plasmolysis takes place much mixed with tonoplast plasmolysis, it may be natural to conclude that the action of  $K^+$  shows the same tendency

Table 11

Previous treatment	Duration in salt solution (hour)	Duration in plasmolyticum	Writer	Chol. & Sand.
A { 1. KCl(m/40)	24	25-35 min.	convex	convex
2. $CaCl_2$ (m/60)	24	25-35 min.	concave	concave
B { 1. KCl(m/6)	3	1 h. 50 min.	tonopl.-convex	concave
2. $CaCl_2$ (m/9)	3	3 hours	convex	convex

as in A 1. From the above comparison it may be said that in the case of Cholodny and Sandkewitsch the microscopical observation was made often at arbitrary duration and their results are insufficient for a comparison of the actions of  $K^+$  and  $Ca^{++}$  with each other. The writer's experiment shows that the general tendency for  $K^+$  to hydrate and  $Ca^{++}$  to dehydrate the protoplasm can be applied in this case too.

### Résumé

1. When the epidemis cell of *Allium cepa* is plasmolysed with a 0.5 mol KCl solution, concave plasmolysis continues longer than hitherto expected, if the material is preserved in healthy condition and its treatment during the experiment is carried out carefully.

2. When KCl is used as the plasmolyticum, the difference of the material and the experimental condition react more sensitively in the plasmolysis form and time than when  $CaCl_2$  or saccharose is used.

3. Judging from the plasmolysis form the liquefying action of  $K^+$  on the cytoplasm increases accompanied by the increase of hydrogen ion concentration.

4. Light has remarkable influence on the plasmolysis form and time. The plasmolysis time is shortened by illumination of the preparation. This is theoretically in accordance with the well-known fact that the light increases the permeability of the protoplasm.

5. In order to have uniform healthy material of *Allium cepa* for the purpose of the experiment on the plasmolysis form, it may be recommended that the bulb or the separated scale leaf be kept cool and wet, or better in cool water. This was confirmed by repeated experiments in the present investigation.

6.  $Ca^{++}$  and  $Al^{3+}$  act antagonistically against  $K^+$  in relation to the plasmolysis form. The plasmolysis time is greatly prolonged by the addition of these ions.

A part of the present study has been carried out at the Laboratory of Plant Physiology of Prof. T. Sakamura, Sapporo, to whom the writer wishes to acknowledge his sincere gratitude for granting him the facilities of the laboratory. The writer expresses his thanks equally to the Japan Society for the Advancement of Cytology, by the grant from which this work was supported.

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## Studies on the Chromosome Numbers in Higher Plants. IV \*

By

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The following is a part of the results obtained from our recent karyological studies. The karyological technique used for the present study was the same as before, as it was necessary to compare the karyological figures previously observed with the present.

Material plants were raised from seeds from the botanical gardens in Europe, to the authorities of which the writer wishes to express his cordial thanks.

### The Mode of Cell Division

The plants described here all show the furrowing process in the mode of the partition wall formation of the pollen mother cells (cf. Sugiura 1936).

### Number of Chromosomes

#### Compositae

Consulting Tischler's Lists of chromosome numbers (1927-38) it seems that there are the two basic numbers 4 and 5 in Compositae. The same numbers were given by Wanscher (1934).

We now enumerate below 3 species of this family whose meiotic chromosomes are 8 each.

*Venidium calandulaceum*. This is grown in S. Africa. In diaphase we see 4 large pairs of chromosomes and 4 small ones. This suggests that the 8 chromosomes consist of 4 + 4.

*Ursinia anthemoides*, *U. speciosa*. These plants are also grown in S. Africa. The meiotic chromosomes of the former are smaller than those of the latter. Formerly the writer studied *U. anethoides* and found the same number of meiotic chromosomes.

Thus we now know that the basic number of *Ursinia* is 8.

#### Plantaginaceae

*Plantago media*. Earlier karyological studies on *Plantago* were made by Němec (1910), Ishikawa (1916), Ekstrand (1918), Sinotô

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(1925), Heitz (1927), Tjebbs (1928), Matsuura and Suto (1935) and McCullagh (1934), the last having carried his investigations particularly far. He concluded that 6 is the basic number in the genus *Plantago* and that 5 and 4 are derived from it.

He found the somatic chromosome number in *P. media* to be 24, while the writer counted 12 meiotic ones. The P.M.Cs are rather large in comparison with the external appearance of the flowers.

### **Acanthaceae**

Of this family little was known karyologically, Gigante's study on *Acanthus mollis* (1929) being the only record. The writer has investigated some species of *Acanthus*, *Justicia*, *Ruellia*, *Thunbergia* and *Dicliptera*, the meiotic chromosome numbers of which were already published. We now publish numbers of meiotic chromosomes in the following two plants.

*Hygrophylla spinosa*. This genus has not been studied karyologically yet. It has 12 spherical meiotic chromosomes, which are about  $0.6 \mu$  in dia., while the P.M.C. is very much larger in comparison, i.e. about  $20 \mu$  in dia..

*Thunbergia reticulata*. Considering our previous studies on the genus *Thunbergia*, it seems that the basic number of chromosome in the genus is 9. The above species has also the same meiotic chromosome number, but the chromosomes are larger than those of *T. alata* (Sugiura 1936).

### **Gesneriaceae**

In spite of the fact that the genus has been cultivated in every country of the world, karyological studies on it were rather few until our studies on *Sinningia*, *Naegelia* and *Saintpaulia* had been published.

*Streptocarpus veitchii*. The writer, having formerly counted 16 meiotic chromosomes in this genus (Sugiura 1936), found the same number in the above species. Generally the P.M.Cs. are smaller by one half those of *Sinningia*.

### **Scrophulariaceae**

*Hebenstreitia virgata*. Some species in the genus were previously studied. The meiotic number of chromosomes is 7 like the former species *H. comosa* and *H. dentata*.

The number 7 is rather rare in this family according to Tischler's Lists of chromosome numbers. It is probably derived from 6, the basic number of the family. At present it is known that genera having 7 meiotic chromosome numbers in the family are *Cymbalaria*, *Alectorolophus*, *Collinsia*, *Veronica*, and *Chaenorphinum*.

*Collinsia bicolor*. Formerly the writer found 7 meiotic chromosomes in *C. candidissima*. This species has also the same number.

*Tetranema mexicana*. The chromosome numbers in the genus have not yet been counted before. It has 10 meiotic chromosomes. The number 10 is rather rare in the family.

Other examples having 10 chromosomes are *Scrophularia* and *Odontites*.

### Solanaceae

*Schizanthus retusus*. *S. pinnatus*. *Schizanthus* belonging to Salpiglossideae has 10 meiotic chromosomes, while the majority of species having the basic number 6.

*Nicandra* has also 10 meiotic chromosomes, according to the karyological studies by de Vilmorin and Simonet (1928) and Janaki-Ammal (1932b).

*S. pinnatus* was first found by E. Marchal (1920) to have 10 meiotic chromosomes. We also found the same number in 3 plants of the genus (Sugiura 1936). The number 11 was also found by the writer in *Browallia* and he believes that these numbers, such as 10 and 11 must be derived from 6, the basic number in this family. Wanscher earlier described that the basic number in the family is 4 rather than 6, judging from his observation of the secondary association of chromosomes (Wanscher 1934). But the writer like most karyologist's thinks that the basic number of chromosomes in the family Solanaceae is 6 for the following reason.

Now we enumerate the chief chromosome numbers(n) hitherto found in the family Solanaceae:

*Browallia* 11, *Capsium* 6, 12, *Datura* 6, 12, 24, *Lycium* 12, *Nicotiana* 9, 12, 24, 48, *Petunia* 7, 14, *Schizanthus* 10, *Solanum* 6, 12 18, 24, 30, 36, 48.

From the above meiotic chromosome numbers we now know that these are derived from 3 and from 4, namely:

3, 6, 9, 12, 18, 24, 30, 36, 48, etc.

Here the number 3, which is not of real existence, but supposed to exist for convenience for an explanation of chromosome numbers, is called the Theoretical Basic Number of chromosomes.

The theoretical basic number 3 is also found in the families Nolanaceae, Scrophulariaceae which are very close to each other serodiagnostically.

Thus we now know that the serodiagnostic and karyological results are the same (cf. Alexnat's serodiagnostic study).



### Nolanaceae

*Nolana* has about 20 species, native to Chile and Peru, many of them being maritime. Formerly Campin (1925), Whyte (1929b) and Datta (1933a) studied *N. atriplicifolia* and *prostrata* and found 12 meiotic chromosomes in them.

We have found the same number in *N. prostrata*, *grandiflora*, *tenella* (Sugiura 1936b).

*Nolana paradoxa*. We now know that this has also 12 meiotic chromosomes. The P.M.Cs. in the second metaphase are rather large (about 20  $\mu$  in dia.). The basic number of chromosomes is 6. The very close family Solanaceae has also the basic number 6.

### Labiatae

*Nepeta*. This has not been studied karyologically hitherto. But the writer now finds the following numbers of meiotic chromosomes in 6 species.

<i>Nepeta Cataria</i>	18	<i>N. Glechoma</i>	9	<i>N. gradiflora</i>	18
<i>N. kokanica</i>	18	<i>N. macrantha</i>	9	<i>N. nuda</i>	9

Thus the basic number should be 9 and the theoretical number 3.

Bushnell (1936) however found 16 chromosomes in diaphase and in homoeotypic equatorial plate of *N. Cataria*, but further confirmation is required.

### Borraginaceae

So far as we know, the chromosome numbers of *Anchusa* are as follows:

Plant names	n	2n	Investigators
<i>Anchusa myosotidiflora</i>	8	12	Strey 1930
<i>A. officinalis</i>	8	12	Smith 1931, 2
<i>A. italica</i>		32	Strey 1930, Smith 1931, 2, Lewitsky 1934.
<i>A. Barrelieri</i>		16	Strey 1930, Smith 1931, 2, Sugiura 1931, 36, Lewitsky 1934.
	8	16	Smith 1931
		18	Strey 1931
<i>A. capensis</i>		16	Lewitsky 1934
<i>A. hybrida</i>		16	Smith 1932
<i>A. procera</i>		16	„ 1931, 2
<i>A. sempervirens</i>		22	Strey 1931
<i>A. ochroleuca</i>		24	Smith 1931, 2
			„ „

Consulting the above list of chromosome numbers of *Anchusa*, we now know that the basic number of chromosomes is 8 and the theoretical basic number 4.

Thus it is easily explained that the meiotic chromosome number

12 which was found in *A. ochroleuca* by Smith would be derived from 4.

There is however a difference between Smith's count and ours in *A. sempervirens*. Smith counted 22 somatic chromosomes, but we counted only 8 meiotic chromosomes.

We have previously studied *Nemophila* in Hydrophyllaceae, *Phacelia* in Phaceliaceae and *Wigandia* in Nameae, and a karyological account of them has already been published (1936). In our former work we showed that there were 9 and 11 meiotic chromosome numbers in *Phacelia*.

And we have now further studied the following 4 species:

	n		n
<i>Phacelia circinata</i>	11	<i>P. dwarecata</i>	10
<i>P. ciliata</i>	9	<i>P. Whitlavia</i>	11

It would appear that there are three kinds of chromosome numbers (n) in the genus *Phacelia*, namely 9, 10 and 11, 9 being the probable basic number. This is derived from 8 which is found in the neighbouring families Boraginaceae, Labiatae and Verbenaceae.

#### **Polemoniaceae**

We previously studied *Collomia* and *Gilia* karyologically, and an account of our findings have already been recorded.

We have studied the following two species and found 9 meiotic chromosomes in each.

<i>Gilia laciniata</i>	n = 9,	<i>G. Liebmannii</i>	n = 9
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According to our studies there are two chromosome numbers in the genus; namely 8 and 9. Flory (1937) studied the genus *Gilia* and found 7 and 9 meiotic chromosomes. He concluded that 9 is the basic number of *Gilia* and 7 is not. 7 must be the basic number of *Ipomopsis* which was proposed by Michaux in 1803. In addition to the chromosome numbers 7 and 9, there is 8, which was found by the writer in *Gilia capitata*. Flory, however, found 9 ( $2n = 18$ ).

In my case 8 chromosomes were found at IM and IA, and I think 8 is probably derived from 9 as a result of the fusion of two of them as in *Lobelia* (Sugiura 1939b).

#### **Melastomataceae**

Formerly Ruys (1925) counted 12 meiotic chromosomes in *Bertolonia marmorata*, and Heitz (1926) found 28–32 somatic chromosomes in *B. marmorata* var. *aenea*. The writer also found 14 meiotic chromosomes in *B. maculata*. As to the chromosome numbers in *Melastoma*, I reported in 1936 that there were 28 meiotic chromosomes. Matsuura and Sutô (1935) also found 56 somatic

chromosomes in *M. sanguineum*. Ruys (1925) in addition to the above genera, counted 12 meiotic chromosomes in *Miconia*, *Memecylon* and 24 in *Triuranthera Winkleri*. Thus we now know that there are two kinds of basic chromosome numbers in the family, viz. 6 and 7. The latter number would probably be the basic number of *Melastoma*.

### Loasaceae

This family contains 5 genera, 3 of which have been previously studied karyologically by the present writer as follows:

<i>Blumenbachia</i>					
<i>hieronymi</i>	12	1936 a, 1936 b.	<i>L. hispida</i>	15	1936 a, 1939 b.
<i>Mentzelia Lindleyi</i>	18	1931, 1936 b.	<i>L. triphylla</i>	14	1936 a, 1939 b.
<i>Loasa aurantiaca</i>	12	1936 a, 1936 b.	<i>L. vulcanica</i>	14	1936 a, 1939 b.

*Loasa ferruginea* and *Erinus* were also studied. The former has 15 meiotic chromosomes and the latter 20. *Cajophora lateritia*. I have counted 8 meiotic chromosomes in this plant. These are very large and seemingly there is no connection between *Loasa* and *Cajophora* karyologically.

According to Schürhoff (1926) the Loasaceae belongs to the Metachlamydeae, and are probably close to the Symplocaceae. But we cannot confirm it karyologically as there have been no chromosome counts of Symplocaceae.

According to Mez, the genus *Loasa* is very near to Cactaceae. The writer formerly determined the somatic chromosomes in certain species of the Cactaceae to be 24 ( $n = 12$ ), while Matsuura and Suto (1935) found 9 in *Zygocactus* and *Neomamillaria*. Considering the fact that the neighbouring genera all have a theoretical basic number of 3, it can be at least said that *Loasa* also has the same theoretical basic number of 3.

### Portulacaceae

Most of the chromosome numbers in this family were unknown with the exception of Tjebbes' count (1928) of 9 meiotic chromosomes, when the writer first undertook to study the chromosomes of *Portulaca grandiflora*.

The results of previous studies of *Portulaca*, *Calandrinia* and *Talinum* are as follows:

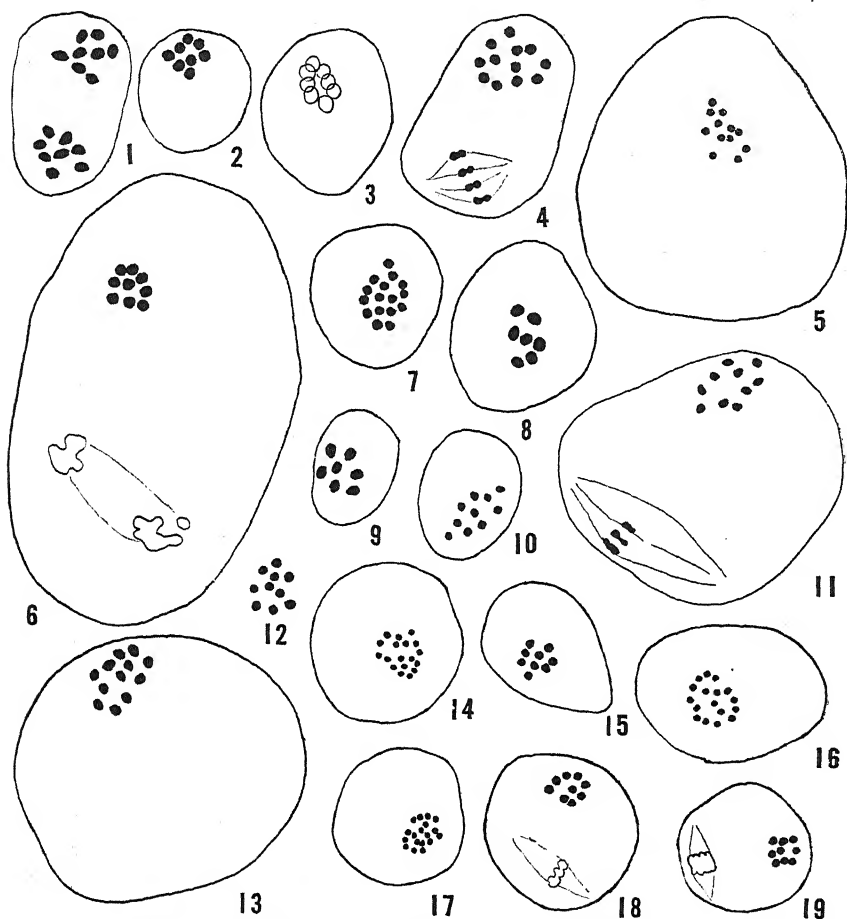
<i>Portulaca grandiflora</i>	9	Tjebbes	1928, Okura 1930.
<i>P. oleracea</i>	9, 27	Hagerup	1932
	27	Blackburn	1934, $2n = 54$ Graner 1936,
		Cooper 1935 a	
<i>P. oleracea</i> var. <i>sativa</i>	26	Sugtura	
<i>P. pusilla</i>	9	„	1936, 37.
<i>P. marginata</i>	18	„	1937

*P. marginata* has 18 meiotic chromosomes. Their arrangement is 11-6-1, which agrees with Thomson's arrangement of corpuscles in stable equilibrium.

As shown above the basic number of *Portulaca* is 9.

*Calandrinia*. This genus has not been studied karyologically. We have counted the meiotic chromosome numbers as follows:

<i>Calandrinia grandiflora</i>	12 (8)	1935, 1936 b.
<i>C. Menziesii</i>	24	1936 a, 1937.
<i>C. procumbens</i>	24	" "
<i>C. speciosa</i>	12	1935, 1936 b.
<i>C. umbellata</i>	10	1936 a, 1936 b.



Figs. 1-19.  $\times 3330$ . 1, *Venidium calandulaceum* IA. 2, *Ursinia anthemoides*. IA. 3, *U. speciosa*. IIM. 4, *Plantago media*. IIM. 5, *Hygrophylla spinosa*. IM. 6, *Thunbergia reticulata*. IIA. 7, *Streptocarpus veitchii*. IM. 8, *Hebenstreitia virgata*. IA. 9, *Collinsia bicolor*. IA. 10, *Tetranema mexicana*. IM. 11, *Schizanthus retusus*. IIM. 12, *S. pinnatus*. IA. 13, *Nolana paradoxa*. IIM. 14, *Nepeta Cataria*. IM. 15, *N. Glechoma*. IM. 16, *N. grandiflora*. IM. 17, *N. kokanica*. IA. 18, *N. macrantha*. IIM. 19, *N. nuda*. IIM.

Formerly we counted 8 meiotic chromosomes in *C. grandiflora*, but now we have confirmed that it is 12 and not 8.

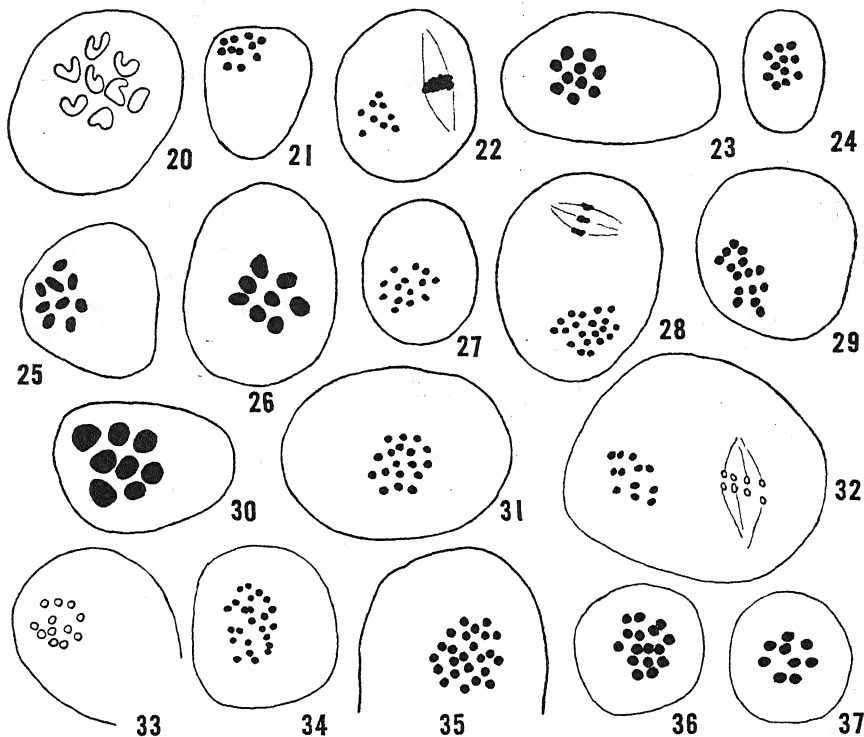
*Calandrinia discolor*  $n = 12$  (9-3). *C. compressa*  $n = 24$  (13-8-3).

The chromosome arrangement of both species agrees with Thomson's arrangement of corpuscles in stable equilibrium as above formula shows (also Figs. 33, 34).

From the above list we now know that 6 is the basic number of the genus and 3 the theoretical basic number.

*Talinum*. There have been no reports since Blackburn found the meiotic number of chromosomes in *T. patens* to be 12 in 1934. We have studied the following 3 plants:

<i>Talinum purpurea</i>	12	1936 a, 1936 b.
<i>T. patens</i> (= <i>paniculata</i> )	12	" "
<i>T. triangulare</i>	24	1938.



Figs. 20-37.  $\times 3330$ . 20, *Anchusa sempervirens*. IA. 21, *Phacelia circinata*. IIM. 22, *P. ciliata*. IIM. 23, *P. dwarecata*. IM. 24, *P. Whitlavia*. IA. 25, *Gilia laciniata*. IM. 26, *G. Liebmannii*. IM. 27, *Bertolonia maculata*. IA. 28, *Loasa ferruginea*. IIM. 29, *L. Erinus*. IIM. 30, *Cajophora lateritia*. IM. 31, *Portulaca marginata*. IM. 32, *Calandrinia grandiflora*. IIM. 33, *C. discolor*. IM. 34, *C. compressa*. IM. 35, *Talinum triangulare*. IM. 36, *Tetragonia crystallina*. IM. 37, *Mesembryanthemum cordifolium*. IM.

Table 1.

Plants investigated	n	IM ( $\mu$ )	IA ( $\mu$ )	IIM ( $\mu$ )	IIA ( $\mu$ )	Fig.
Compositae						
<i>Venidium calandulaceum</i>	8		1.25×0.8			1
<i>Ursinia anthemoides</i>	8		1.0			2
<i>U. speciosa</i>	8			1.0		3
Plantaginaceae						
<i>Plantago media</i>	12			0.75		4
Acanthaceae						
<i>Hygrophylla spinosa</i>	12	0.6				5
<i>Thunbergia reticulata</i>	9				0.75	6
Gesneriaceae						
<i>Streptocarpus vetchii</i>	16	0.75				7
Scrophulariaceae						
<i>Hebenstreitia virgata</i>	7		1×0.7			8
<i>Collinsia bicolor</i>	7		0.75			9
<i>Tetranema mexicana</i>	10	0.63				10
Solanaceae						
<i>Schizanthus retusus</i>	10			0.625		11
<i>S. pinnatus</i>	10			0.625		12
Nolanaceae						
<i>Nolana paradoxa</i>	12			0.87		13
Labiatae						
<i>Nepeta Cataria</i>	18	0.4				14
<i>N. Glechoma</i>	9	0.625				15
<i>N. grandiflora</i>	18	0.5				16
<i>N. kokanica</i>	18		0.4			17
<i>N. macrantha</i>	9			0.5		18
<i>N. nuda</i>	9			0.5		19
Borraginaceae						
<i>Anchusa sempervirens</i>	8					20
Hydrophyllaceae						
<i>Phacelia circinata</i>	11			0.5		21
<i>P. ciliata</i>	9			0.37		22
<i>P. dwarecata</i>	10	0.75				23
<i>P. Whitlavia</i>	11					24
Polemoniaceae						
<i>Gilia laciniata</i>	9	1.25×0.8				25
<i>G. Liebmannii</i>	9	1.5 ×1.0				26
Melastomataceae						
<i>Bertolonia maculata</i>	14		0.5			27
Loasaceae						
<i>Loasa ferruginea</i>	15			0.62		28
<i>L. Erinus</i>	20			0.375		29
<i>Cajophora lateritia</i>	8	1.5				30
Portulacaceae						
<i>Portulaca marginata</i>	18	0.5				31
<i>Clandrinia grandiflora</i>	12					32
<i>C. discolor</i>	12	0.625				33
<i>C. compressa</i>	12	0.45				34
<i>Talinum triangulare</i>	24	0.75				35
Aizoaceae						
<i>Tetragonia crystallina</i>	16	0.75				36
<i>Mesembryanthemum cordifolium</i>	9	0.875				37

Thus the basic number in this genus is 12 at present.

Summarizing the above we now know that the basic number in *Portulaca* is 9, while that in *Calandrinia* and *Talinum* is 12. According to Pax, Portulacaceae and Aizoaceae are closely related. Karyologically this can be recognized too, for they both have a theoretical basic number of 3.

### Aizoaceae

*Tetragonia crystallina*. The n-chromosome number in the species is 16, just the same as in other species of *Tetragonia*. There have been few such plants in this family as *Tetragonia* with 16 meiotic chromosomes to date. For example,

<i>Aizoon canariense</i>	16	<i>Tetragonia expansa</i>	16
<i>Mollugo verticillata</i>	32	<i>T. echinata</i>	16

Karyological studies on *Mesembryanthemum* to date have proved that it has a basic chromosome number 9. The *Mesembryanthemum* (in the broad sense) has more than 800 species, while Aizoaceae contains 1100 species and 23 genera. Thus at present there are two kinds of chromosome numbers in this family; 9 and 8. But discussion of them should be postponed until a karyological investigation of the remaining genera has been made.

The results of the present work are summarized in Table 1.

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# **Zytologische Untersuchungen über die intramuralen Ganglienzellen des Verdauungstraktes. Über die Ganglienzellen des menschlichen Darmes, mit besonderer Berücksichtigung auf die Nisslsubstanz**

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## **I. Einleitung**

Die mikroskopischen Untersuchungen der Ganglienzellen des autonomen Nervensystems wurden bis jetzt zum größten Teil mittels Silberimprägnationsmethode ausgeführt. Neuerdings hat ITO (1936) und ITO und NAGAHIRO (1937) die intramuralen Ganglienzellen des Verdauungstraktes mit der gewöhnlichen histologischen und zytologischen Untersuchungsmethode erforscht und konnte Golgiapparat, Mitochondrien, Pigmentgranula u.a. nachweisen, dabei haben sie besonders in der Anordnung des Golgiapparates eine Eigentümlichkeit dieser Ganglienzellen wahrgenommen. Zugleich haben sie auch die Nissl'sche Substanz der intramuralen Ganglienzellen eingehend beschrieben. Die Nissl'sche Substanz der vegetativen Ganglienzellen wurden von relativ zahlreichen Autoren untersucht, wie von VAS (1892), MARINESCO (1898), CARPENTER und CONEL (1914), PING (1921), TAKAKUSU (1924), CLARK (1926), HERZOG (1926), WATZKA (1929), PENITSCHKA (1929), KUNTZ (1929), BRADSHAW (1930), EINARSON (1933), INGERSOLL (1934) u.a. Dieselbe der intramuralen Ganglienzellen des Darmkanals wurde von TAKAKUSU (1924), OSHIMA (1929), STÖHR (1931) und NOMURA (1931) erforscht. Aus den Ergebnissen der genannten Autoren geschlossen, zeigt die Nisslsubstanz sowohl der verschiedenen sympathischen Ganglien als auch der Darmgeflechte, wie die Zusammenfassung von ITO und NAGAHIRO (1937), zwei Eigentümlichkeiten. Erstens ist die Nisslsubstanz meistens fein granulär und im Zytoplasma diffus verteilt. Sie bildet in der Regel keine grobschollige Körperchen wie die Tigroidschollen der zerebrospinalen Ganglienzellen. Zweitens bildet sie gewöhnlich eine periphere und häufig auch eine perinukleäre Anhäufungszone. Diese zwei Besonderheiten haben ITO (1936) bei den intramuralen Ganglienzellen des menschlichen Wurmfortsatzes und ITO und NAGAHIRO (1937) bei denselben des Rattendarmes bestätigt.



Ziemlich grobschollige Nisslsubstanz wurde aber von STÖHR (1930) bei den Ganglienzellen des AUERBACH'schen Plexus der Katze und von ITO (1936) bei denselben des menschlichen Wurmfortsatzes selten wahrgenommen. Solche Erscheinung scheint jedoch sehr selten sich zu finden.

Es wurde von Autoren häufig vorgenommen, die vegetativen Ganglienzellen nach ihrer Größe und nach den Beschaffenheiten der Fortsätze in verschiedene Typen einzuteilen. Dazu bietet die Beschaffenheit der Nisslsubstanz auch ein wichtiges Merkmal dar. Schon haben CLARK (1926), BRADSHAW (1930), NOMURA (1931), EINARSON (1933), INGERSOLL (1934) u.a. nach den Beschaffenheiten der Nisslsubstanz die Ganglienzellen der sympathischen Ganglien in verschiedene Typen eingeteilt.

Die Frage, ob zwischen den Ganglienzellen des Plexus myentericus und den des Plexus submucosus, vom morphologischen und physiologischen Standpunkt betrachtet, irgendein Unterschied liegt oder nicht, ist heute noch eine interessante. TAKAKUSU (1924) hat auf den Unterschied der Nisslsubstanz zwischen dem Plexus myentericus und dem Plexus submucosus aufmerksam gemacht, was ITO (1936) bei menschlichem Wurmfortsatz und ITO und NAGAHIRO (1937) bei Rattendarm nicht bestätigen konnten. Diese Forscher haben aber andererseits erkannt, daß die Beschaffenheit des Golgiapparates nach den Ganglienzellen der beiden Geflechte einen merklichen Unterschied zeigt.

Die vorliegende Untersuchung haben wir vorgenommen, um die Nisslsubstanz der intramuralen Ganglienzellen des Darmes beim menschlichen Material genau zu studieren, weil bei Menschen die Nisslsubstanz der genannten Ganglienzellen, die ITO's Untersuchung beim menschlichen Wurmfortsatz ausgenommen, bis jetzt eingehend kaum studiert worden ist. Es ist sehr schwierig, das frische und gesunde Material aus dem Menschenkörper herauszunehmen. Die von uns benutzten Darmstücke wurden alle von gesunden Hingerichteten im lebendfrischen Zustand herausgenommen, so daß die pathologischen sowie postmortalen Veränderungen vollkommen ausgeschaltet werden konnten.

## II. Material und Methode

Die uns zur Verfügung gestellten Untersuchungsmaterialien waren Dünn- und Dickdärme, die aus sechs, lebendfrischen, männlichen Hingerichteten abstammten. Näheres ist in folgender Tabelle 1 angegeben. Das Material erwies sich sowohl makroskopisch wie mikroskopisch als gesund. Im lebendfrischen Zustand wurden die

Materialien in 10% Formol und Formol-Alkohol (Formol 1 Teil: 95% Alkohol 9 Teile) fixiert, und in Zelloidin eingebettet. Wir haben sie 7–10  $\mu$  dick geschnitten und mit dem SPIELMEYER'schen Ver-

Tabelle 1

Nr.	Alter	Geschlecht
1.	23	Mann
2.	26	„
3.	45	„
5.	32	„
11.	33	„
12.	34	„

fahren gefärbt. Nämlich wir haben die Zelloidinschnitte aus dem 70% Alkohol in die 0,1% wässrige Toluidinblaulösung eingetaucht und unter zweimaliger Erwärmung gefärbt. Nach dem Erkalten wurden die gefärbten Schnitte zuerst im destillierten Wasser, dann kurz im 70% Alkohol abgespült, darauf im 95% Alkohol unter Mikroskopierung

differenziert. Die fertigen Schnitte kamen durch absoluten Alkohol in Xylol, und wurden im Balsam eingeschlossen. Außerdem haben wir die Schnitte zum Teil in Hämatoxylin (HANSEN's)-Eosin gefärbt.

Alle Figuren sind mit dem ABBE'schen Zeichenapparat auf Objekttischhöhe und zwar mit ZEISS' Immersion 1/12 und Komp.-Ok. 12 gezeichnet.

### III. Eigene Befunde

Die intramuralen Ganglienzellen des Darmes stellen wechselnde Größe und Form dar. Im allgemeinen sind die Zellen des Plexus myentericus am größten und häufig längs gestreckt, während die des Plexus submucosus meistens rundlich gestaltet sind. Dies scheint auf das Verhältnis zurückzuführen zu sein, daß die Ganglienzellen des Plexus myentericus im engeren Raum zwischen den zwei Muskelschichten zahlreich zusammengedrungen sind. Der Kern liegt in der Regel stark exzentrisch im Zytoplasma. Diese Tatsache haben ITO (1936) und ITO und NAGAHIRO (1937) als eine wichtige Eigenschaft der vegetativen Ganglienzellen betont. Gewöhnlich sind die Ganglienzellen in der Darmwand in Ganglien des Plexus submucosus und Plexus myentericus verteilt. VAN ESVELD (1928) hat berichtet, daß die Ganglienzellen von Typus des AUERBACH'schen Plexus bei Katzendarm in der Ringmuskulatur vorkommen können. Der gleiche Befund wurde auch von EVANS und UNDERHILL (1923), STÖHR (1930), NOMURA (1930), OKAMURA (1930), TAKAYASU (1933, '34, 35a, b,) u.a. angeführt. Nach NOMURA (1930), REISER (1932), SATO (1935) und ITO (1936) kommt bei menschlichen Wurmfortsatz der Nervenplexus mit den Ganglienzellen nicht selten innerhalb Ring- und Längsfaserschicht der Muskulatur vor. ITO hat sogar aufmerksam gemacht, daß dabei die eigentliche Lokalisationsstelle des Plexus myentericus, der Zwischenraum zwischen den beiden Muskelschichten, häufig frei von Plexus gelassen sein kann.

Innerhalb der Lamina muscularis mucosae hat REISER (1932) bei dem menschlichen Wurmfortsatz die Ganglienzellen gefunden. MASSON (zit. nach REISER (1932)) hat erwähnt, daß es zwischen dem Epithel und der Lamina muscularis mucosae den periglandulären Nervenplexus gibt. Neuerdings hat ISHISAWA (1939) berichtet, daß er die Ganglienzellen in Lamina propria des menschlichen Dünndarmes nicht so selten gefunden hat.

In der vorliegenden Untersuchung haben wir bei dem menschlichen Darm außer dem eigentlichen Plexus myentericus im Zwischenraum zwischen dem Ring- und Längsfaserschicht der Muskulatur und dem Plexus submucosus in der Tela submucosa, welche beide immer am stärksten entwickelt sind, noch die Ganglienzellen in Lamina propria, Lamina muscularis mucosae, innerhalb der beiden Muskelschichten und in Tunica serosa bemerkt. Die Ganglienzellen in der Lamina propria sind im allgemeinen klein und treten meistens vereinzelt auf (Fig. 1). Die Ganglienzellen innerhalb der beiden Muskellagen stellen gewöhnlich keine große Gruppen dar, sondern sind vereinzelt oder in kleinen Gruppen aus etwa 2–3 Zellen vorhanden. Die Ganglienzellen in der Lamina muscularis mucosae sind klein, an Zahl gering (Fig. 2). In der Tunica serosa sind auch die Ganglienzellen selten vorhanden.

Über die Frage, ob der Nervenplexus in der Lamina propria vorhanden ist oder nicht, gehen die Meinungen der Autoren auseinander. MASSON hat angenommen, daß ein Nervenplexus, von ihm mit periglandulärem Nervenplexus benannt, vorhanden ist. Dagegen hat ISHISAWA angegeben, daß das Vorkommen des Nervenplexus in der Lamina propria ihm zweifelhaft ist, aber die in der Lamina propria liegenden Ganglienzellen durch die Nervenfaserbündel, welche die Muscularis mucosae durchbohren, mit den Ganglien des MEISSNER'schen Plexus verbunden sind. Nach unserer eigenen Beobachtung der menschlichen Darmwand ist die Häufigkeit des Vorkommens der Ganglienzellen in der Lamina propria sehr klein, sie ist viel kleiner als die in der Muscularis mucosae; es war uns unmöglich, hier mit Sicherheit zu entscheiden, ob ein besonderer Nervenplexus in der Lamina propria der menschlichen Darmwand vorhanden ist oder nicht.

Wie oben schon erwähnt, ist die Nisslsubstanz der vegetativen Ganglienzellen von zahlreichen Autoren auseinandergesetzt worden ist. Im Vergleich mit den Ganglienzellen des zerebrospinalen Ganglion zeigt die Nisslsubstanz der genannten Ganglienzellen einige Besonderheiten, was schon von Autoren, wie HERZOG (1926), OSHIMA (1929), NOMURA (1931), DE CASTRO (1932), ITO (1936), ITO und NAGAHIRO (1937) u.a., aufmerksam gemacht worden ist. Nach ITO

und NAGAIHIRO kann man aus den Befunden der Autoren zwei Eigentümlichkeiten der Nisslsubstanz in den vegetativen Ganglienzellen schließen. Erstens ist die Nisslsubstanz in der Mehrzahl der Fälle fein granulär, und im Zytoplasma diffus verteilt. Sie bildet niemals grobschollige Körperchen (diffuse Form). Zweitens bildet sie meistens eine periphere (Randtigroid oder peripherer Ring) und eine perinukleäre dichtere Anhäufungszone (perinukleärer Ring). In seiner Arbeit der intramuralen Ganglienzellen des menschlichen Wurmfortsatzes hat ITO (1936) selbst diese Eigentümlichkeiten nahezu vollkommen bestätigt, doch hat er dabei selten solche Ganglienzellen wahrgenommen, welche verhältnismäßig grobe Nisslschollen führen.

In der vorliegenden Untersuchung der intramuralen Ganglienzellen des menschlichen Darmes haben wir nahezu vollkommen die oben angeführten Besonderheiten der Nisslsubstanz der vegetativen Ganglienzellen bestätigt. Nach unserer eigenen Beobachtung ist die

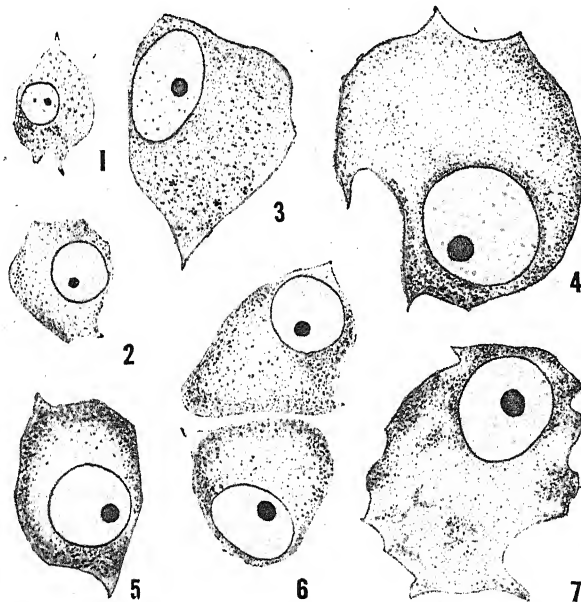


Fig. 1-7. Die Ganglienzellen aus der menschlichen Darmwand; 1 aus Lamina propria; 2 aus Lamina muscularis mucosae; 3-7 aus Plexus submucosus. Fixiert in Formol-Alkohol, mit Ausnahme der Ganglienzelle in Fig. 7, welche allein in 10% Formalin fixiert wird, gefärbt nach SPIELMEYER mit 0,1% Toluidinblau. ca. 1700 $\times$ .

Nisslsubstanz der intramuralen Ganglienzellen des menschlichen Darmes immer fein granulär und im ganzen Zytoplasma diffus verteilt, wie in den Figuren leicht ersehen. Die gut ausgeprägten Tigroidschollen, welche man z.B. in den motorischen Vorderwurzelzellen des Rückenmarkes findet, trifft man hier nicht. Obwohl die Nisslsubstanz im ganzen Zytoplasma diffus verteilt ist, kann man in bestimmten Stellen des Zytoplasma die dichtere Anhäufung

der Nisslgranula finden, welche meistens eine Zone darstellt. Diese Anhäufungszone zeigt in der Regel keine scharfe Kontur, sie geht meis-

tens ganz allmählich in die Umgebung über. Am häufigsten finden wir solche dichtere Anhäufungszone in der Peripherie der Zelle vor. Sie stellt meistens verhältnismäßig schmale, bei Toluidinblaufärbung dunkel angefärbte Zone dar; wenn sie sich auf die ganze Randzone der Zelle erstreckt, so entsteht ein sogenannter „peripherer Ring“ (Fig. 4, 5, 6, 9, 10). Er ist aber oft unvollkommen, indem er stellenweise unterbrochen ist. Außerdem finden wir häufig die Anhäufungszone perinukleär; wenn sie vollkommen den Kern umgibt, so entsteht ein sogenannter „perinukleärer Ring.“ Dieser ist auch schmal, liegt immer unmittelbar auf der Kernmembran (Fig. 4, 9, 10). Dieser perinukleäre Ring ist aber öfters vermißt, doch sind die Ganglienzellen ohne peripherer Ring viel seltener, wie von ITO (1936) verwiesen. Weiter stellt zuweilen die dichtere Anhäufung der Nisslgranula ganz verschieden große unregelmäßig gestaltete Flecken dar, welche in verschiedenen Stellen des Zytoplasma zu finden sind (Fig. 7, 16, 17). Die Kontur solcher Flecken ist aber meist nicht scharf, so daß die Flecken ganz anders als die genuinen Tigroidschollen aussehen. ITO (1936) hat, wie oben referiert, bei der Untersuchung der intramuralen Ganglienzellen des menschlichen Wurmfortsatzes die relativ grobschollige Nisslkörperchen enthaltenden Ganglienzellen beschrieben und abgebildet.

Nach den Ergebnissen der vorliegenden Untersuchung des menschlichen Darmes müssen wir die Angabe von ITO (1936) bei der Untersuchung des menschlichen Wurmfortsatzes, daß man durch die Beschaffenheiten der Nisslsubstanz die Ganglienzellen des Plexus myentericus und des Plexus submucosus kaum unterscheiden kann, zum Teil korrigieren. Die Beschaffenheit der Nisslsubstanz ist nach den beiden Plexus wesentlich nicht verschieden. Doch haben wir in dieser Untersuchung entdeckt, daß in den mit Toluidinblau gefärbten Nisslpräparaten die Ganglienzellen des Plexus myentericus und des Plexus submucosus leicht voneinander unterschieden werden können. Bei der Nisslfärbung mit Toluidinblau färben sich die Ganglienzellen des Plexus submucosus viel intensiver als die des Plexus myentericus; sie sind gegen die Differenzierung mit Alkohol viel resistenter als die letzteren, so daß sie in den Nisslpräparaten im allgemeinen viel dunkler als die Ganglienzellen des Plexus myentericus aussehen (Fig. 3–7). Dies beruht höchstwahrscheinlich zum Teil auf der dichteren Anordnung der Nisslsubstanz in Zellen des Plexus submucosus und zum Teil auf der Beschaffenheit des Zytoplasma selbst. Nach TAKAKUSU (1924) sind die Nisslkörperchen bei den Ganglienzellen des Plexus myentericus feiner als bei den des Plexus submucosus. Dieses Ergebnis konnten wir nicht bestätigen. Der Befund von ITO und NAGAHIRO (1937) bei den Ganglienzellen

des Rattendarmes, daß die Beschaffenheit des Golgiapparates nach den Ganglienzellen des Plexus myentericus und des Plexus submucosus verschieden ist, ist im Vergleich mit dem Unserigen bei der Nisslsubstanz sehr interessant. Das Ergebnis, daß die Beschaffenheit der Ganglienzellen des Plexus myentericus und Plexus submucosus so klar verschieden ist, scheint uns sehr bedeutsam und wichtig.

Wir können also bei der Nisslfärbung die Ganglienzellen des Typus von Plexus submucosus und die des Typus von Plexus myentericus unterscheiden; der erstere Typus zeichnet sich durch im allgemeinen dunkel gefärbtes Zytoplasma und der letztere durch im allgemeinen helles Zytoplasma aus. Aus diesem Standpunkt betrachtet, gehören die kleinen Ganglienzellen in der Lamina propria und der Lamina muscularis mucosae dem Typus von Plexus submucosus, die in der Tunica serosa und den Muskelschichten dem Typus von Plexus myentericus an.

Die Einteilung der vegetativen Ganglienzellen in verschiedene Typen wurde von früher vorgenommen. Dafür wurden Form des Ganglienzellen bzw. Beschaffenheiten der Fortsätze, die Größe des Zelleibes und die Beschaffenheiten der Nisslsubstanz als Merkmale der Einteilung benutzt.

Die Einteilung der vegetativen Ganglienzellen in verschiedene Typen nach den Beschaffenheiten der Nisslsubstanz wurde von CARPENTER und CONEL (1914), CLARK (1926), BRADSHAW (1930), NOMURA (1930), INGERSOLL (1934) u.a. vorgenommen. CLARK hat sogar 7 Typen unterschieden. NOMURA hat neuerdings die intramuralen Ganglienzellen der Darmwand von Taube nach den Beschaffenheiten der Nisslsubstanz in drei Typen eingeteilt. Bei dem ersten und dritten Typus sind die Nisslsubstanz diffus im Zytoplasma verteilt, obwohl sie in der Umgebung des Kerns etwas dichter angehäuft sind; bei dem ersten ist die Anordnung der Nisslgranula viel dichter, so daß er viel dunkler aussieht. Bei dem zweiten Typus findet man die periphere und perinukleäre Anhäufung der Nisslgranula. In der vorgehenden Untersuchung der intramuralen Ganglienzellen des Darmes haben ITO (1936) und ITO und NAGAHIRO (1937) solche Einteilung nach den Beschaffenheiten der Nisslsubstanz nicht vorgenommen. In der vorliegenden Untersuchung haben wir aber diese an den intramuralen Ganglienzellen des menschlichen Dünn- und Dickdarmes getan.

Wir müssen zuerst den Typus von Plexus myentericus und den Typus von Plexus submucosus abtrennen. Weil diese beiden Typen, wie oben erwähnt, einen wesentlichen Unterschied haben, so daß man sie einzeln behandeln muß. Die beiden Typen werden wieder je in

einzelne Untertypen eingeteilt. Dabei haben wir besonders die Eigenschaften des peripheren und perinukleären Ringes berücksichtigt.

(I) Die Ganglienzellen des Typus von Plexus submucosus.

1) Der erste Typus. Bei den Zellen von diesem Typus sind die feinen Nisslgranula gleichmäßig diffus im Zytoplasma verteilt und bilden niemals besondere Anhäufung (Fig. 3).

2) Der zweite Typus. Bei diesem Typus sind die Nisslgranula diffus im Zytoplasma verteilt, aber sie bilden sowohl den peripheren als auch den perinukleären Ring (Fig. 4).

3) Der dritte Typus. Wir finden bei diesem Typus nur peripheren Ring (Fig. 5, 6).

4) Der vierte Typus. Bei diesem Typus bilden die diffus verteilten Nisslgranula hie und da undeutliche fleckweise Anhäufungen mit unscharfer Grenze. Bei ihm finden wir auch die Neigung zur Bildung der peripheren und perinukleären Anhäufung (Fig. 7).

(II) Die Ganglienzellen des Typus von Plexus myentericus.

1) Der erste Typus. Bei diesem, wie der erste des Plexus submucosus, sind die ebenso feinen Nisslgranula ganz gleichmäßig diffus im Zytoplasma verteilt und bilden keineswegs besondere Anhäufung (Fig. 8).

2) Der zweite Typus. Bei diesem, wie der zweite des Plexus submucosus, sind die Nisslgranula diffus im Zytoplasma verteilt, aber sie bilden in der Peripherie des Zytoplasma und auf der Kernmembran je eine vollkommene ringförmige Anhäufung aus (peripherer und perinukleärer Ring) (Fig. 9 und 10).

3) Der dritte Typus. Dieser Typus besitzt sowohl den peripheren als auch den perinukleären Ring, aber die beiden Ringe sind unvollkommen ausgebildet, so daß sie an beiden Enden des Kerns und des Zytoplasma unterbrochen sind (Fig. 11 und 12).

4) Der vierte Typus. Bei diesem findet man nur den peripheren Ring. Dieser Typus entspricht dem dritten des Plexus submucosus (Fig. 13).

5) Der fünfte Typus. Die Ganglienzellen von diesem Typus haben unvollständigen peripheren Ring; dieser ist an beiden Enden des Zytoplasma unterbrochen (Fig. 14).

6) Der sechste Typus. Bei diesem findet man die dichte Anhäufung der Nisslgranula ringförmig auf der Kernmembran (perinukleärer Ring) und in deren Nachbarschaft (Fig. 15).

7) Der siebente Typus. Bei diesem bemerkt man an den Nisslgranula die starke Neigung zu Ansammeln in Flecken; in unbestimmten Stellen des Zytoplasma findet man wechselnd große fleckweise Anhäufungen der Nisslgranula ohne scharfe Grenze. Der sonstige Zytoplasmateil ist ungemein hell (Fig. 16 und 17).

Wir haben in der Darmwand niemals die Ganglienzellen ohne Nisslgranula wahrgenommen, im Gegensatz zur Angabe von HERZOG (1926) bei pathologischen Fällen.

Die Frage, ob die Nisslsubstanz in Fortsätzen vorhanden ist oder nicht, ist hier auch kurz auseinanderzusetzen. Bei zerebrospinalen Ganglienzellen dringt die Nisslsubstanz, wie bekannt, in Dendriten ein, aber nicht in Neuriten. Neuerdings hat ITO (1936) angegeben, daß die Nisslsubstanz sich im proximalen Teil fast aller Fortsätze der intramuralen Ganglienzellen des menschlichen Wurmfortsatzes befindet. Er konnte aber bei einer Ganglienzelle solch einen Fortsatz bemerken, bei welchem die Nisslsubstanz vollständig fehlt und dessen Wurzelteil einen dem Ursprungskegel ähnlichen hellen Plasmahof führt. Er hat somit vermutet, daß es sich bei diesem Fortsatz um Neurit dieser Ganglienzelle handele, obwohl die Frage, ob man bei den vegetativen Ganglienzellen die Neuriten und Dendriten voneinander unterscheiden kann oder nicht, heute noch bestritten ist (STÖHR (1928), KURÉ (1934) u.a.). Wir haben in der vorliegenden Untersuchung im Wurzelteil der Fortsätze der intramuralen Ganglienzellen des menschlichen Darmes bald reichliche Nisslsubstanz und bald nahezu keine bemerkt, und es gelang uns leider nicht, die Fortsätze der längeren Strecke nach zu verfolgen. Also kamen wir über die Frage der Nisslsubstanz in Fortsätzen der intramuralen Ganglienzellen hier nicht zu entscheidenden Ergebnissen.

Die Mehrkernigkeit der sympathischen Ganglienzellen haben einige Autoren, wie TAKAKUSU (1924), TERPLAN (1926), HERZOG (1926), STÖHR (1928), WATZKA (1929), PENITSCHKA (1929), MÜLLER (1931) u.a. erwähnt. Nach WATZKA (1929) nimmt die Mehrkernigkeit bei normalen Ganglienzellen mit dem Alter ab und bei den mehrkernigen Ganglienzellen sind die Kerne niemals kleiner als bei den einkernigen. WATZKA hat in dem Ganglion der menschlichen Samenblase eine 14-kernige Ganglienzelle gefunden; STÖHR (1928) hat berichtet, daß die in der Umgebung der Samenblase und Prostata befindlichen sympathischen Ganglienzellen sich durch ihre Mehrkernigkeit auszeichnen. HERZOG (1926) hat bei den sympathischen Ganglien des Grenzstranges 6- und 8-kernige Ganglienzellen beobachtet. ITO (1936) und ITO und NAGAHIRO (1937) konnten beim je menschlichen Wurmfortsatz und Rattendarm die mehrkernigen Ganglienzellen nicht finden. ITO hat vermutet, daß bei den Ganglienzellen in der Magendarmwand die Mehrkernigkeit äußerst selten oder nur ausnahmsweise aufzutreten scheint.

Wir haben aber bei unseren Präparaten des menschlichen Dünn- und Dickdarmes nicht selten zweikernige Ganglienzellen, und zwar



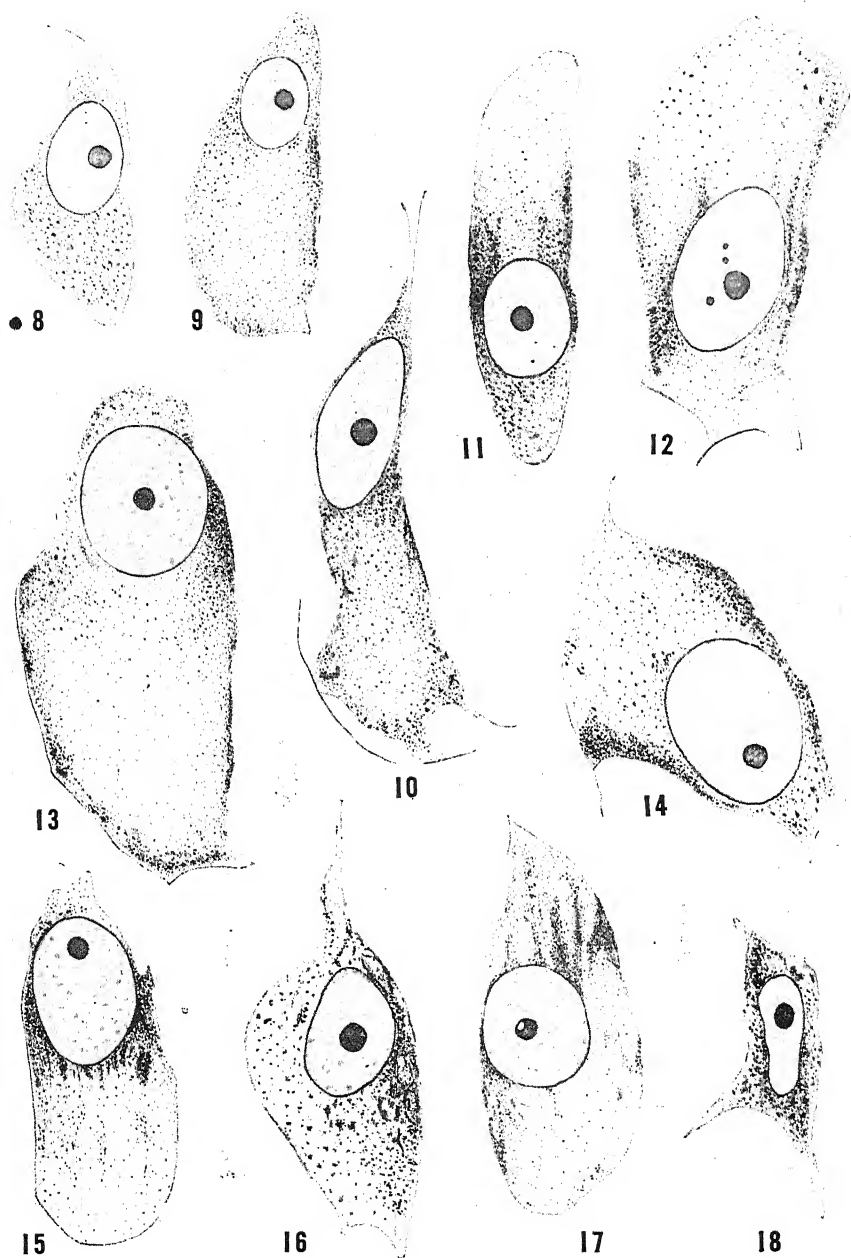


Fig. 8-18. Die Ganglienzellen aus der menschlichen Darmwand; 8-18 aus Plexus myentericus. Fixiert in Formol-Alkohol, mit Ausnahme der Ganglienzellen in 14 und 16, welche in 10% Formalin fixiert werden, gefärbt nach SPIELMEYER mit 0,1% Toluidinblau. ca. 1700 $\times$ .

in der Dünndarmwand (Plexus submucosus) eines 26 jährigen Mannes sogar eine vierkernige gefunden. Die Häufigkeit des Vorkommens der mehrkernigen Ganglienzellen ist bei einzelnen untersuchten Fällen und bei dem Dünn- und Dickdarm fast gleich.

Das Auftreten der pyknotischen Ganglienzellen in vegetativen Ganglien wurde von einigen Autoren, wie WATZKA (1929), FÜRBRINGER (1909) (zit. nach WATZKA), ITO (1936) und ITO und NAGAHIRO (1937), berichtet. WATZKA hat beschrieben, daß die pyknotischen Ganglienzellen bei allen Altersstufen, besonders zahlreich im höheren Alter anzutreffen sind. ITO (1936) begegnete bei seiner Untersuchung über Wurmfortsatz des Menschen pyknotische Ganglienzellen besonders oft im Plexus myentericus, aber äußerst selten im Plexus submucosus; er ist zur Meinung gekommen, daß der auf den Plexus myentericus wirkende Druck der Muskulatur als verursachender Moment dieser Zellen anzusehen sei.

In der vorliegenden Untersuchung konnten wir die ITO's Befunde bestätigen; die pyknotischen Ganglienzellen, welche sich durch schmalen Zellkörper und länglich ovalen, dunkel erscheinenden Kern charakterisieren, wurden in der Dünn- und Dickdarmwand des Menschen fast ausschließlich im AUERBACH'schen Plexus gefunden. Den pyknotischen Ganglienzellen fehlt die Nisslsubstanz nicht; die Nisslgranula sind bei ihnen im ganzen Zytoplasma regellos verteilt, zum Teil in zahlreiche kleine Flecke angehäuft (Fig. 18).

Es handelt sich bei den pyknotischen Ganglienzellen, wie uns scheint, wahrscheinlich um die degenerierenden Ganglienzellen. Wie von ITO angenommen, scheinen die Ganglienzellen der Darmwand im physiologischen Zustand mit dem Alter nach und nach der Degeneration anheimzufallen, um schließlich zugrunde zu gehen, was mit dem Herabsteigen der Darmfunktion in höheren Alter in inniger Beziehung steht.

#### IV. Zusammenfassung der Ergebnisse

1. In der vorliegenden Untersuchung haben wir die intramuralen Ganglienzellen des im lebendfrischen Zustand fixierten menschlichen Dünn- und Dickdarmes aus 6 ganz gesunden Hingerichteten hauptsächlich in den nach SPIELMEYER mit Toluidinblau gefärbten Nisslpräparaten zytologisch studiert. Dabei haben wir außer der Nisslsubstanz noch die Verteilung der Ganglienzellen in verschiedenen Schichten der Darmwand, die Mehrkernigkeit der Ganglienzellen, das Auftreten der pyknotischen Ganglienzellen u.a. beobachtet.

2. Die Ganglienzellen werden nicht nur im eigentlichen Plexus myentericus und Plexus submucosus, sondern auch in der Lamina propria mucosae, Lamina muscularis mucosae, in den beiden Schi-

chten der Tunica muscularis und in der Tunica serosa, also in allen Schichten der Darmwand vorgefunden.

3. Die Nisslsubstanz der intramuralen Ganglienzellen des menschlichen Darmes stellt "diffuse Form" dar, d.h. die feinen Nisslgranula sind im ganzen Zytoplasma diffus verteilt, ohne die Tigroidschollen bildend. Dabei wird aber häufig bemerkt, daß die Nisslgranula besonders in der Peripherie des Zytoplasma und perinukleär dichter anhäufend den sogenannten „peripheren“ und „perinukleären Ring“ bilden; den ersteren trifft man viel häufiger als den letzteren an, sie können aber oft simultan in einer Zelle vorkommen. Selten findet man solche Ganglienzellen, welche die verschieden großen und unregelmäßig geformten, den Tigroidschollen ähnlichen Flecke der dichteren Anhäufung der Nisslgranula führen.

4. Obwohl die Beschaffenheit der Nisslsubstanz bei dem Plexus myentericus und bei dem Plexus submucosus wesentlich nicht verschieden ist, kann man die Ganglienzellen der beiden Geflechte in den Nisslpräparaten leicht und klar unterscheiden; in den genannten Präparaten sehen die Ganglienzellen des Plexus submucosus im allgemeinen viel dunkler aus als die des Plexus myentericus, indem jene mit Toluidinblau im ganzen intensiver angefärbt sind als diese.

5. Also kann man die intramuralen Ganglienzellen der Darmwand nach der Nisslfärbung in zwei Typen, den Typus von Plexus myentericus und den von Plexus submucosus, einteilen. Der erstere sieht in den Nisslpräparaten hell und der letztere dunkel aus. Die Ganglienzellen in der Lamina propria und der Lamina muscularis mucosae, welche beide im allgemeinen sehr klein sind, gehören zu dem Typus von Plexus submucosus und die in den beiden Schichten der Tunica muscularis und in der Tunica serosa zu dem von Plexus myentericus an.

6. Wir haben weiter hauptsächlich nach dem Vorkommen und der Beschaffenheit des peripheren und perinukleären Ringes die Ganglienzellen des Typus von Plexus submucosus wieder in 4 Typen und die des Typus von Plexus myentericus in 7 Typen eingeteilt.

7. Gegen die Frage, ob durch das Vorhandensein und Fehlen der Nisslsubstanz in Fortsätzen die Neuriten und Dendriten der intramuralen Ganglienzellen unterschieden werden können, hat die vorliegende Untersuchung zu keiner entscheidenden Lösung geführt.

8. Die zweikernigen Ganglienzellen werden bei allen untersuchten Fällen sowohl im Plexus myentericus als auch im Plexus submucosus nicht selten vorgefunden; wir haben sogar eine vierkernige wahrgenommen.

9. Die pyknotische Ganglienzellen werden bei jedem Falle bemerkt, zwar fast ausschließlich im Plexus myentericus. Sie

scheinen höchstwahrscheinlich für das physiologische allmähliche Zugrundegehen der intramuralen Ganglienzellen des Darmes mit dem Alter zu sprechen.

Zum Schluß sprechen wir Herrn Dr. G. TAKAHASHI<sup>†</sup>, Vorstand des pathologischen Institutes des Mantetsu-Hospital zu Hsin-King und Herrn Dr. K. KISO, Vorstand der Augenklinik desselben Hospital, für ihre freundliche Hilfe bei Ansammlung des Untersuchungsmaterials unseren herzlichsten Dank aus.

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Chromosome Studies in Cyperaceae, VI. Pollen development and additional evidence for the compound chromosome in *Scirpus lacustris* L.<sup>1)</sup>

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(With 14 text-figures and 30 photomicrographs)

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The pollen development in Cyperaceae has long been noticed by its speciality that a pollen mother cell undergoes meiosis in the normal way resulting in quartet nuclei of which only one develops to a pollen grain nucleus while the rest degenerate; therefore, only one pollen grain instead of four arises from one pollen mother cell. The earliest cytological study in this family by Elfving (1879) has established the characteristic position of Cyperaceae in the cytological fields in Angiosperms. His work on the pollen development of *Heleocharis palustris* as well as the succeeding many investigations such as by Strasburger (1884), Juel (1900), Stout (1912), Heilborn (1918, 1922), Piech (1924a, b, 1928), and recently by the writer (1939c) have revealed the characteristic features in the pollen development in this family; yet several questions are being left undissolved.

The present material, *Scirpus lacustris* L., was first investigated by Piech (1924a) on the pollen development, later by Håkansson (1928) and Kostrionkoff (1930) on the chromosome number and recently by the writer on the special chromosome conditions in three types, i.e. normal self-colored, and the other two variegated (1937a, b, 1938, 1939a). The writer's material plant was differed from that investigated by Håkansson and Kostrionkoff in its chromosome number having a peculiar compound chromosome (cf. 1937a, 1938), that is three chromosome numbers i.e.  $2n = 38$ ,  $2n = 40$ , and  $2n = 42$  were found respectively in *typicus*, *pictus* and in *zebrinus*, while only  $2n = 42$  was reported by other investigators. *Typicus*<sup>2)</sup> and *pictus* have the peculiar large compound chromosomes in the somatic cells,

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2) Three types of *Scirpus lacustris* L. are verified by Prof. Honda; self-colored type as *S. lacustris* L. var. *typicus* Honda, two variegated types as *S. lacustris* L. var. *typicus* Honda f. *pictus* Honda and *S. lacustris* L. var. *Tabernaemontani* Trautv. f. *zebrinus* Makino. Throughout this paper these three types will be referred to as *typicus*, *pictus*, and *zebrinus* respectively.

two in the former and one in the last. In the previous papers (1937a, b, 1938, 1939a) it was predicted that the peculiar large chromosome may be equivalent to three small chromosomes and this has partly been proved by the chromosome behaviour in the meiotic division of one variegated type *pictus* as the 1-3 small chromosomes have paired with one large chromosome in the first meiotic metaphase. Thus the peculiar large chromosome in the writer's materials has been considered as a sort of compound chromosome. In the present paper an additional evidence for this compound chromosome in *typicus* as well as some contributions for the pollen development will be given.

### Material and Methods

Material used in the present investigation was taken from the same plant which was used in the previous papers of 1937a and b, and of 1938 as *Scirpus lacustris* L. and as *S. lacustris* L. var. *typicus* Honda, respectively.

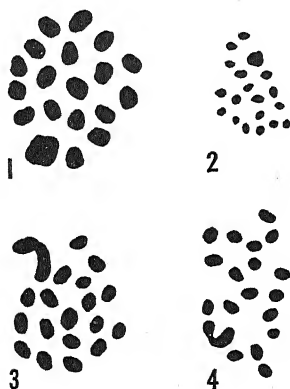
The inflorescences were fixed with Carnoy's fluid (absolute alcohol 3 part, glacial acetic acid 1 part) for a few hours. They are preserved in 75% alcohol, after being washed in abs. alcohol. From the preserved inflorescence the anther sacs were taken out with needles and stained with aceto-carmin. The cover-glass was sealed with "Valap," a newly made sealing agent in our laboratory, a mixture of vaseline (2 part), lanolin (2 part) and paraffin (1 part). Being the melting point of the valap is about 45°C, it is convenient in the practical use to keep the valap in the thermostat, and seal the borders of the cover-glass with glass bar when necessary (Tanaka and Suita, 1939). Overstained or old preparations were revived with a few drops of 50% acetic acid after being removed the sealing valap with chloroform.

### Observations

Reduction division. The maturation division of *typicus* has been already reported in the first paper of this series, but the details were missing, which will be reported on this occasion.

**I-Metaphase.** As already reported (1937a, b, 1938) the *typicus* has 38 chromosomes in diploid of which 36 chromosomes are small and 2 are larger. In the meiotic prophase (diakinesis) the compound chromosome pair is attached to the nucleolus (cf. 1938). The existence of the compound chromosomes in *typicus* is suggestable enough to encounter to some irregularities in their meiotic pairing, so the fairly high number of the PMC's, i.e. 218 PMC's in polar view and 1383 PMC's in side view, have been observed, but the result has fallen

short of expectation. Formation of the equatorial plate in the I-metaphase was absolutely normal, and chromosome association was quite regular, showing 18 bivalents and one compound chromosome pair (fig. 1: photo 1.). However a small degree of irregularities were met with in later stages.



Figs. 1-4.  $\times 2200$ . Chromosome complements in I-M (1), II-M (2) and in the primary pollen nuclear division metaphase (3, 4); the compound chromosome is present in each case.

**I-Anaphase.** Separation of chromosomes in the first anaphase was quite regular referring to the small chromosome pairs, but was irregular in the compound chromosomes, sometimes have separated regularly i.e. simultaneously with the small chromosome pairs, but in most cases that is in 65 PMC's out of 73 observed (89.04%) they were lagging behind. And very now and then, already in this stage, more than one fibre attachment could be seen in the compound chromosomes; this becomes more distinct in the following stage when they have formed the chromosome bridges.

**I-Telophase.** In this stage the small chromosomes have already separated to the poles; however, some structural hybridities in the chromosomal constitution have been met with solely in the compound chromosomes. In 959 PMC's (89.54%) out of 1071 observed all chromosomes have separated to the poles, while the compound chromosomes were always delayed in their arrival to the poles. In the rest 112 PMC's structural hybridities have been found. As seen from table 1, chromosome bridges which were always composed of

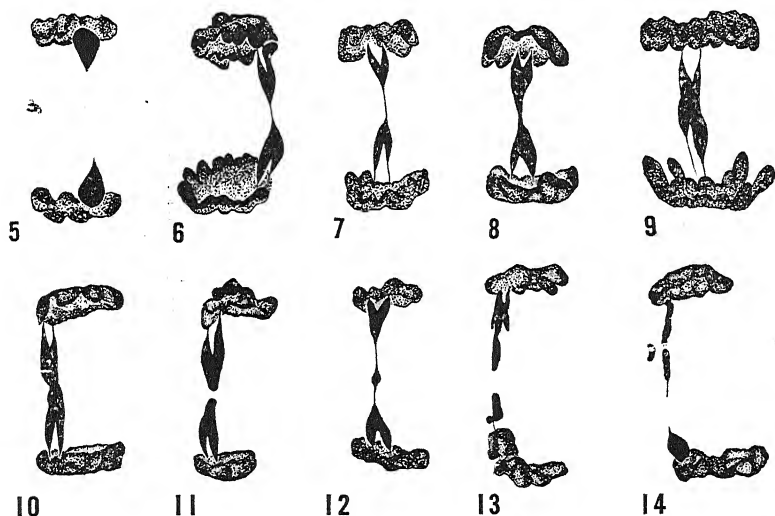
Table 1. Chromosomal abnormalities in meiosis of *typicus*.

Stage	I-Anaphase	I-Telophase	II-Telophase	P-I-Telophase
Normal	8	959 (89.54%)	284 (98.27%)	219 (99.96%)
Abnormal { bridge { lagging	65 { 0 { 65 (89.04%)	112 { 110 (10.27%) { 2 (0.19%)	5 { 4 (1.38%) { 1	1 { 0 { 1
Total	73	1071	289	220

the compound chromosome were observed in 110 PMC's out of 1071 observed (10.27%) and the lagging compound chromosome was observed in 2 PMC's (0.19%). It will be of noteworthy that these abnormalities have occurred, without any exceptions, in the compound



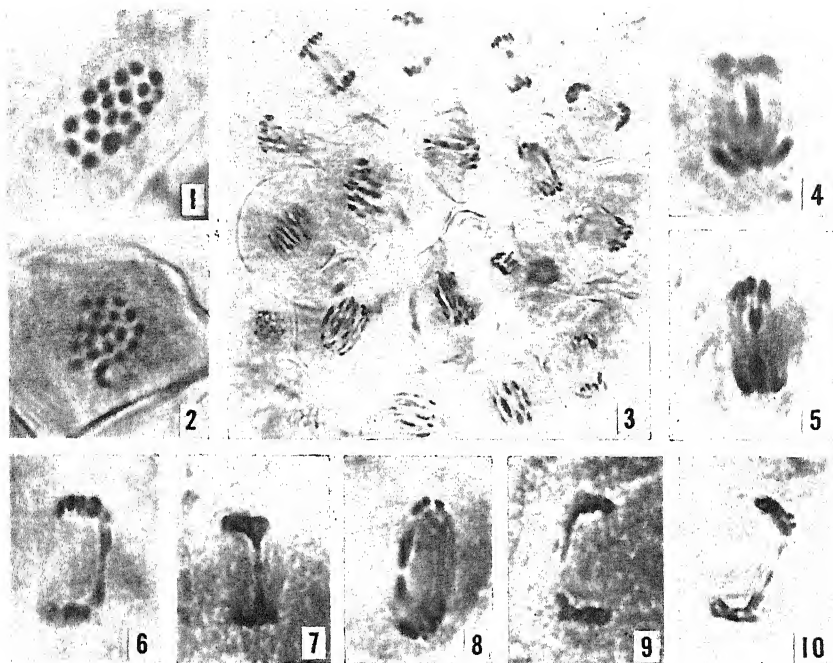
chromosomes solely. In the chromosome bridges three types have been seen. The most striking, common feature throughout the types is that the chromosome bridges are having always clearly more than one fibre attachment. In the first type, the chromosome bridge was consisted of two chromatin masses, each of which having a sagittate form with two fibre attachments, and of a feeble chromatin thread connecting the daughter chromosomes. This first type is most prevalent; 100 bridges out of 110 cases belonged to this type (figs. 6, 7, 8: cf. photos. 5, 6.). In the second type both ends of the bridge have the same sagittate form with two fibre attachments likewise in the first case but is different in the central region of the chromatin thread connecting the daughter chromosomes, where a small chro-



Figs. 5-14.  $\times 2200$ . Chromosome separation in I-T. 5, normal separation. The compound chromosome is always retarded in separation. 6-14, various chromosome bridges in I-T. See in the text.

matin mass could be distinguished. Out of the 110 bridges, 6 cases belonged to this type (fig. 12: photo. 7.). In the last type, chromosome bridges have irregularly broken in the connecting chromatin thread, giving sometimes a chromosomal fragment and sometimes an unequal division of the chromatin masses (figs. 11, 13, 14: photos. 8, 9, 10.). As seen from the table 1, decrease in the percentage of the irregularities can be seen as the stage of nuclear division proceeds from the I-anaphase to the I-telophase. This fact shows that the laggards are to be included into the polar mass eventually, and the small per cent of the chromosome bridges are left behind by their retardation in separation.

**I-Interkinesis.** From the considerable abnormalities seen in the I-telophase, it will be a matter of course, if any irregularities occur in the I-interkinesis. Generally, if any chromosomes or chromosomal fragments are left in the cytosome, i.e. when they are too late in their separation to be included in the daughter nuclei, they sometimes form various kinds of micronuclei or have been left in the cytosome retaining their form in the stainable condition. Total number of 769 PMC's of this stage were carefully observed, but they



Photos. 1-10.  $\times 1700$ . *Scirpus lacustris* var. *typicus*. 1, I-M. 2, P-M. 3, chromosome separation in I-A, I-T. Notice retardation of the compound chromosome in separation. 4-10, various chromosome bridges in I-T.

have contained neither micronuclei nor visible fragments, no chromatin thread still persisting between the daughter nuclei. This fact may be interpreted with high degree of certainty that the laggards or chromosomal fragments which were always consisted of the compound chromosome or have derived from the chromosome bridges, have been included in both polar masses as the stage proceeds.

**II-Metaphase.** From the irregular separation of the compound chromosomes which formed the chromosome bridges in the I-telophase, some differences between the compound chromosomes in this stage may be found owing to the occurrence of rather high percentage of the chromosome bridge. Unfortunately rather small number of the II-metaphase plates i.e. only 28 plates have been observed, all of

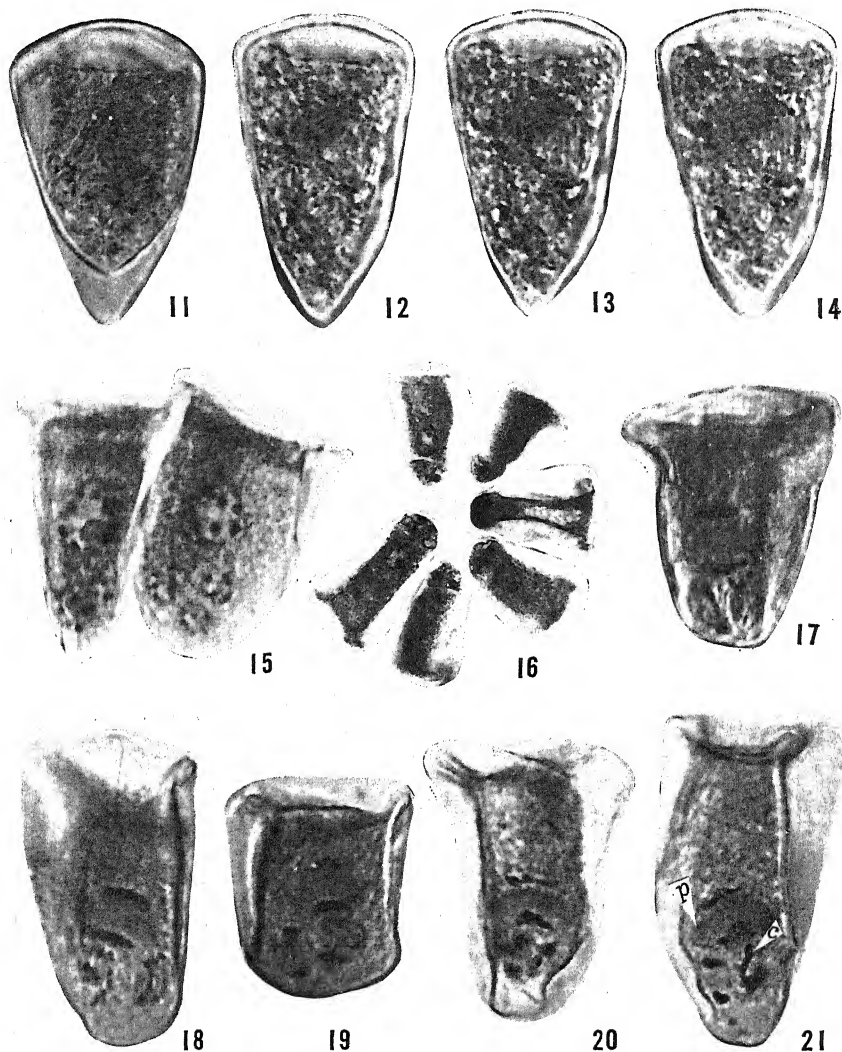
which were normal having 18 small chromosomes and a large compound chromosome, and no distinguishable differences among the compound chromosomes have been found.

**II-Telophase.** Chromosome separation in the II-telophase was rather regular than that in the I-telophase. As was seen in the I-telophase fairly high percentage of the compound chromosomes have proved that they were possessing more than one fibre attachment, hence in the II-telophase it would be expected to meet with some chromosomal irregularities, at least, in the compound chromosome in as nearly percent as in the I-telophase. But the observations of 289 daughter nuclei in the II-telophase have shown that the chromosome separation in this stage has rather normally proceeded. Out of 289 daughter nuclei, in only 5 nuclei (1.73%) chromosomal irregularities have been met with and in the rest (98.27%) no kinds of irregularities could be seen. These irregularities in this stage are of two kinds; the one is the formation of chromosome bridge (4 cases) and the other, occurrence of the laggards (1 case). It is very interesting that in the first, these irregularities are always take place in the compound chromosomes only, and in the second, the percentage of the occurrence of the chromosome bridge has much decreased in the II-telophase than in the I-telophase.

**II-Interkinesis.** 300 PMC's were observed in this stage of which all cells have proved to be normal, having no chromosomal fragments nor chromosomes being left in the cytosome. In the early stage spindle fibers were formed among the quartet nuclei (photos. 11-14.). Photomicrographs (12-14) were taken at successive three foci, showing these spindle fiber formations in one PMC. Then the quartet nuclei have moved together to the inner corner of the PMC and the spindle fibers are disappeared. Here the arrangement of the PMC's in anther will be referred to; in Cyperaceae, several PMC's arrange in one concentric circular layer in a cross section of an anther, giving a wedge-shape to each PMC (cf. photos. 15, 16.). Out of the quartets which are of the same size when they are first formed, only one nucleus which is situated in the outermost position in the PMC grows larger and soon this enlarged nucleus which becomes a pollen nucleus has been separated by a septum from the three other daughter nuclei in the inner corner of the PMC. Among the three nuclei in the corner feeble septa seem to exist. These three nuclei degenerate finally, and the original PMC grows to a young pollen grain instead of four.

**Pollen grain division.** Adult pollen grain in Cyperaceae have three nuclei excepting the three degenerating ones in the corner. Thus in Cyperaceae two successive pollen nuclear divisions have to

take place before germination; the second division occurs only in generative nucleus. In the present investigation only the first nuclear division has been observed.



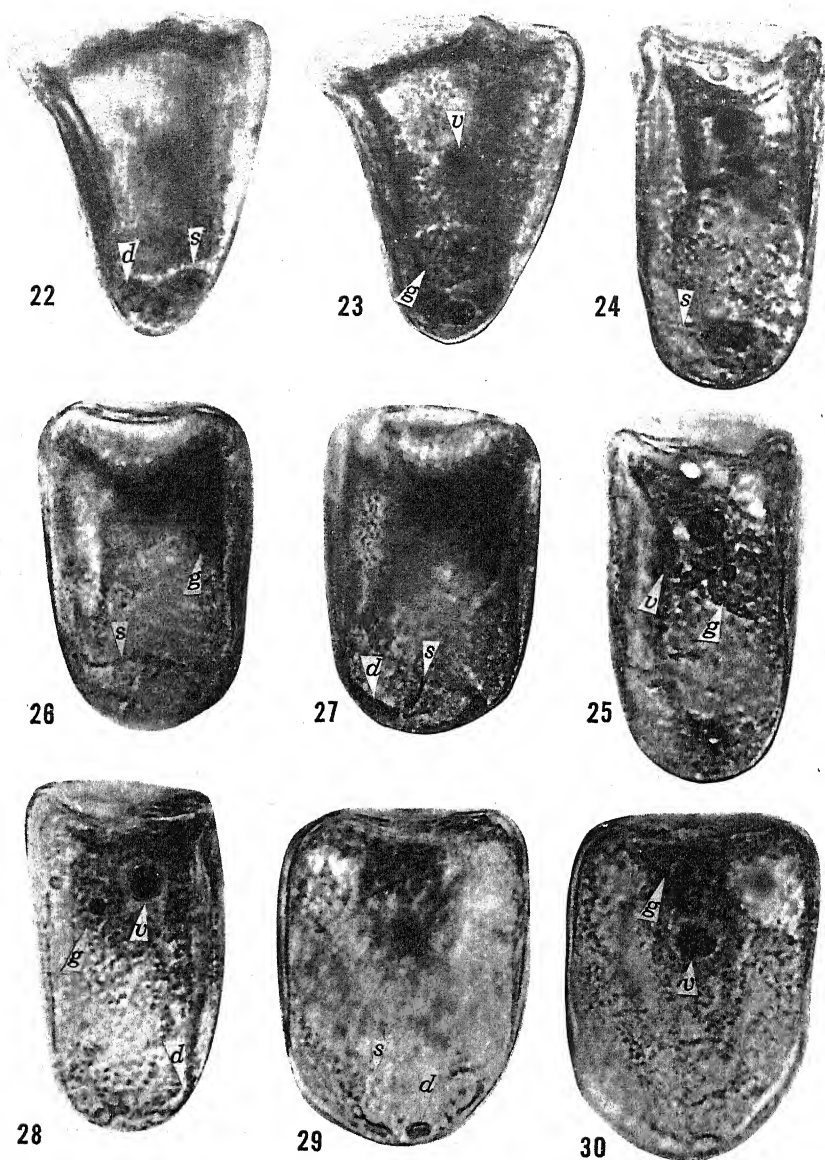
Photos. 11-21. Pollen development. 11, spindle formation among the three daughter nuclei. 12-14, photos taken at three successive foci of a PMC, showing spindle formation among quartet nuclei. 15, PMC's in prophase of the pollen division. Quartet nuclei are dividing simultaneously. The left is the side view, the right the upside. 16, PMC's in early prophase of the primary pollen nuclear division. Septum is seen between the pollen nucleus and the degenerating three nuclei in the corner. 17-20, telophase of the pollen nuclear division. 17, 18, earlier stages, degenerating micronuclei in the corner are dividing, here also the compound chromosomes are retarding in their separation. 19, 20, later stages, the phragmoplast has appeared and spread along the surface of the inner daughter nucleus (generative nucleus). 21, late telophase; one compound chromosome is excluded from the nuclei.  $\times 1400$ .

C-compound chromosome; P-phragmoplast.

**P-I-Metaphase.** In the first pollen grain metaphase, 19 chromosomes were clearly counted (fig. 3, 4: photo. 2.) in 63 pollen grains. The equatorial plate formation in the P-I-metaphase, in side view were observed in 374 pollen grains and was proved to be quite normal. One of the 19 chromosomes is larger than the rest and undoubtedly this is the compound chromosome. A slight size difference, however, could be distinguished among the compound chromosomes. The three nuclei which finally degenerate, in the corner, have got into the nuclear division simultaneously with the pollen nucleus (photos. 15-23.). In the P-I-metaphase of the pollen nucleus, these three nuclei also have formed equatorial plates and have divided by themselves (photos. 17-23.). Therefore it is evident that the three small nuclei proved to possess the ability of dividing by themselves.

**P-I-Telophase.** The pollen nucleus divides normally and two daughter nuclei arise; the one in the outer position grows larger and becomes a vegetative nucleus and the other in the inner position becomes a generative one. Observations of 220 pollen grain divisions have proved that the separation of the chromosomes in the P-I-telophase had proceeded quite normally. In this stage, the phragmoplast has appeared first in the center of the telophasic spindle (photos. 17-21.), and then it has spread to both sides parallel to the surface of the generative nucleus, and finally a generative cell plate was formed by means of the union of the phragmoplasts. The chromosome separation in the three nuclei in the corner was rather delayed and very often chromosome bridges which were always composed of the compound chromosome have been observed. This delayed separation, especially in the compound chromosomes would be caused by the limited space in the corner. Nevertheless these three nuclei which bearing the fatal to degenerate, have divided resulting in 6 micronuclei. In 219 pollen grains out of the 220 observed pollen grains in this stage, no kinds of abnormalities have been met with, but in one pollen grain one compound chromosome has been left in the cytosome near the inner part of the pollen grain (photo. 21.). This fact shows that in spite of the facts that either in the I-interkinesis or in the II-interkinesis no chromosomal remainders have been observed, very rarely the compound chromosome has been left behind in the telophase separation and excluded from the daughter nuclei into the cytosome.

By the union of the phragmoplasts, as already mentioned, a generative cell plate is formed, and has been separated with the pollen grain cytoplasm. The generative cell plate as well as the vegetative nucleus move to the outer position and at last the former takes the place of the latter. Meanwhile the 6 micronuclei which have arisen



Photos. 22-30. Pollen development (cont.). 22, 23, photos taken at two foci, showing a septum between the pollen nuclei and the six degenerating micronuclei in the corner. In photo 23, a generative cell plate is seen. 24, 25, photos taken at two foci, advanced stage; a generative cell plate has moved upwards. 26, 27, photos taken at two foci, showing the septa in the corner. 28, more advanced stage, micronuclei are adhering to the PMC's wall. 29, 30, photos taken at two foci; generative cell plate has already moved peripherally. Remnants of the septa among the micronuclei in the corner can be seen.  $\times 1400$ .

*D*-degenerating nucleus in the corner; *G*-generative cell; *S*-septum;  
*V*-vegetative nucleus.

after the nuclear division of the three nuclei could not grow to 6 independent nuclei and formed 3 restitution nuclei which were pushed against the cell wall by and by, and finally degenerated. However the septa among the three nuclei in the corner, and those between the pollen nuclei and the degenerating micronuclei stand still fairly until later stages. Microphotos. 24-29 show the remainders of these septa in the pollen grain.

### Comments

**Chromosome bridge.** The occurrence of chromosome bridges in meiosis and in somatic mitosis is of great significance, since these chromosome bridges must have derived from the chromosome with double attachments. The chromosome bridges in the present case are different from that hitherto reported in various other materials (cf. Tanaka, 1938) in two main points; first, in their frequent occurrence in the first meiotic division, and in the second, the special sagittate form with clear double attachments in each end. These two facts will be sufficient to elucidate that the large chromosomes in *typicus* are the compound chromosome. The so-called "Sammel-chromosome" or "multiple chromosome" in the animal kingdom has generally occurred only in the meiotic division, generally only in the oögenesis (Federley, 1938), hence they are different from the compound chromosome in the present material which is bearing always their compound feature in every stages of all life cycle. In this point the compound chromosome in the present case and that recently found in *Carex oxyandra* Kudo (Tanaka, unpublished data) are quite different in their constant compound feature. It will be certainly correct that the very occurrence of the chromosome bridge in the compound chromosomes in *typicus* is showing the polykinetic condition of the compound chromosomes. Here we come across a question that why the chromosome bridge does not occur in the II-telophase and in the P-I-telophase as nearly percent as in the I-telophase; in the I-telophase chromosome bridges have occurred in 10.27% PMC's observed, while in the II-telophase only in 1.38% and none in the P-I-telophase. This fact may be interpreted more likely to consider that the behaviour of the compound chromosome in mitosis is governed by one principal kinetochore and that more than one kinetochore are to function only when some structural changes such as translocation, crossing-over, or segmental interchanges have occurred in the compound chromosomes than to consider that there are some unstable chromosome among the compound ones in meiosis, so that chromosome bridge does not occur in mitosis. If the behaviour of the compound chromosomes in mitosis is not managed by a principal

kinetochore, high percent of abnormalities should occur in every mitosis. As a matter of course, in order that the behaviour of the compound chromosome should be controlled by a principal kinetochore, fusion of chromosomes must be complete which may be true in *typicus*. The above argument, however, is reached from the standpoint that the compound chromosome is made by fusion of three small chromosomes; on the contrary, isn't it possible to consider the phenomenon in reverse way, that is, by the *de novo* formation of kinetochore in large not-compound chromosomes beside the normal kinetochore? If the last prediction get the mark, this will also throw some light on the chromosome evolution. This question, however, requires more profound future investigations.

Pollen development in Cyperaceae has long been investigated by several investigators in four genera, i.e. in *Heleocharis* (Elfving, 1879; Strasburger, 1884; Håkansson, 1927-'28), in *Scirpus* (Piech, 1924a, b; 1928; Håkansson 1927-28), in *Carex* (Juel, 1900; Stout, 1912; Heilborn 1918, 1920; Tanaka, 1939b) and in *Fuirena* (Suessenguth, 1921).

**Septum formation in the pollen grain.** As for the question that any septa between the pollen nucleus and the three micronuclei which have been pushed into the inner corner of the PMC as well as those among the three micronuclei in the corner would arise or not, some different views have been held by several authors owing to the difficulties in observations. These views can be classified into three classes; that is, (a) in the first, septa arise, but they soon disappear (Elfving, 1879), (b) in the second, septa arise (Strasburger, 1884; Juel, 1900; Stout, 1912; Håkansson, 1927-'28; Tanaka, 1939b) and (c) in the last the septa formation was denied (Piech, 1924a, b, 1928; Suessenguth, 1921). Even in the same species various views have been offered. For example, in *Heleocharis palustris*, Elfving (1879) held the first, Strasburger (1884) the second, while Piech (1924b) the third view. The last author, however, has reported that the pollen nucleus and the micronuclei in the corner were separated by the secondarily formed callose membrane. Probably he has missed the septa among the micronuclei in the corner hindered by the difficulties in observation. It is most appropriate that the septa formation between the pollen nucleus and the three micronuclei in the corner as well as that among the three micronuclei in the corner is normal. In *Scirpus lacustris* L. Piech (1924a) has recognized no visible cell plate nor plasmic membrane among the quartet nuclei. But the present writer could seen clear septa among them. Concerning the septa formation among the quartets in two genera *Carex* and *Scirpus*, at least, the present writer hold the first and second opinions.



In the present case, in the interstage of prophase and metaphase of the primary pollen nuclear division, a septum is formed between the pollen nucleus and the three small nuclei which finally degenerate and at the same time or somewhat later septa are also formed among the three small nuclei. The retardation in the septum formation in Cyperaceae, especially that of among the three small nuclei in the corner will be due to the irregular wedge shape of the PMC's. By and by these septa, excepting that between the pollen nuclei and the three micronuclei in the corner, were destroyed as the stage proceeds and at last they disappear.

**The fate of the three small nuclei in the corner.** As for the course of degeneration of the three small nuclei which have been pushed into the narrow corner after the second division, three different views have been held. Elfving (1879) and Strasburger (1884) in *Heleocharis palustris* held the opinion that the three small nuclei may be absorbed in the cytosome, while Wille (1886) held the opinion that the three small nuclei may fuse with the pollen nucleus. On the other hand other investigators, like Håkansson (1928), Piech (1928), Juel (1900), Tanaka (1939c), have considered that these three nuclei degenerate owing to the limited space. Except for Juel (1900) and the present writer (1939c) all other investigators have not observed the nuclear division in the three small nuclei in the corner. But the observations in *Carex acuta* (Juel, 1900), *Carex grallatoria* var. *heteroclita* (Tanaka, 1939c) and in *Scirpus lacustris* var. *typicus* (this paper) have proved that these small nuclei have an ability of dividing by themselves. Piech (1928), in *Heleocharis palustris*, has reported that one side spindle had been formed in the nuclear division in these three small nuclei in the corner but showed no division of the chromatin substances. In the present material in nearly almost pollen mother cells the nuclear division took place in these three nuclei and have resulted in two daughter nuclei in each nucleus giving 6 micronuclei in the corner, but in *Carex grallatoria* var. *heteroclita* the nuclear division in the corner was proceeded rather irregularly resulting in 3-6 daughter nuclei after division (Tanaka, 1939c). Between these daughter nuclei in the corner no septum has been formed, and finally they fuse two by two resulting in original three chromatin masses and at last they were pushed against the cell wall to die. This degenerating course of the nuclei in the corner may be due partly to the special wedge shape of the pollen mother cells which is forced by the arrangement of the pollen mother cells in the anther, and partly due to the ill nutritional condition which is caused by the lack of the periplasmodium in the anther sac as Piech (1924) has suggested.

**Pollen nucleus.** In Angiosperms, the situation of the pollen nucleus is deviated from the center of the pollen grain and it divides itself either out- or inwards according to its position. In some exceptional cases (*Sambucus*, *Strychnos*) the primary pollen nuclear division takes place in the central region of the pollen grain (cf. Wulff and Maheshwari, 1938). In Cyperaceae, this point has been left unknown. Piech (1928) in *Heleocharis palustris* has reported that the primary pollen nuclear division took place in the center of the pollen grain, while in *Carex grallatoria* var. *heteroclita*, and in the present material the situation of the nucleus is deviated inwards. The pollen nucleus is situated in the center when looking a pollen mother cell as a pollen grain as a whole, but is eccentric in a pollen grain after the mother cell was divided into two parts, i.e. the pollen nucleus and the three small nuclei, the former situates inwards in the pollen grain and the primary pollen nuclear division takes place outwards.

**Generative nucleus.** By the primary pollen nuclear division a generative and a vegetative nuclei arise; the former situates in the inner and the latter in the outer region. A generative nucleus grows to a generative cell plate; a generative cell plate is formed by means of the union of the phragmoplasts which first appear in the central region of the telophasic spindle and then spread parallel to the surface of the generative nucleus, just like the cases in *Heleocharis palustris* by Piech (1928) and in *Carex grallatoria* var. *heteroclita* by the writer (1939c). Juel (1900), in *Carex acuta*, has predicted that a generative cell plate will be formed by condensation of cytoplasm around the generative nucleus, but he has observed no distinguishable process in its development. A generative cell plate in Angiosperms is usually situated along the cell wall and is not free in the cytoplasm. Piech (1928) has reported in his material, *Heleocharis palustris*, that a generative cell plate is formed free in the cytosome. Generally in Angiosperms, with exception of Cyperaceae, it is quite difficult to determine whether a generative cell plate is lying along the inner cell wall or the outer, as the quartets set free (cf. Wulff and Maheshwari, 1938). In Cyperaceae the original pollen mother cell becomes a pollen grain as a whole, the determination of the position of the generative cell plate is quite easy; at first, it is clearly along the inner cell wall.

### Résumé

The maturation division and pollen development of *Scirpus lacustris* L. var. *typicus* Honda have been described. *Typicus* has

two compound chromosomes in diploid which pair quite normally in meiosis. In the I-telophase this compound chromosome pair forms very often chromosome bridge which always have a sagittate form showing clearly more than one fibre attachment.

The compound chromosome found in *typicus* is different from that found in the animal kingdom in two points, i.e. firstly, it shows the compound feature throughout the life cycle, and secondly, it gives clear direct evidence for the compound feature showing more than one fibre attachment in the bridge formation.

Thus the previous prediction that the compound chromosomes first found in *typicus* and then found in *pictus* may be equivalent to three small chromosomes, is to some extent fulfilled.

Quartet nuclei are of the same size when they are first formed and then move together inwards of the PMC, but soon one of them which is situated in the outer-most position of the PMC grows larger and the rest are pushed to an inner corner of the PMC.

In the interstage of the prophase and metaphase of the primary pollen nuclear division, a septum is formed between the pollen nucleus and the three small nuclei which finally degenerate, and at the same time or somewhat later, septa are also formed among the three small nuclei. The three small nuclei proved to possess the ability of dividing by themselves.

A pollen nucleus undergoes mitosis in the normal way to give rise a generative nucleus inwards and a vegetative one outwards. A generative cell plate is formed by means of the union of the phragmoplasts which appear at first in the center of the telophasic spindle of the primary pollen nuclear division. It is, at first, situated along the wall which divides the pollen nuclei and the micronuclei in the corner.

Between the daughter nuclei in the corner which have arisen by the nuclear division of the three small nuclei no septum has been formed, and finally they fuse two by two which are at last pushed against the cell wall to die.

The septa formed in an inner corner of the PMC seem finally to destroy.

Here the writer wishes to express his best thanks to Prof. Y. Sinotô, by whose suggestion these studies were started, for his valuable advice throughout the course of the work.

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## Studies on the Chromosome Numbers in Higher Plants. V\*

By

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Received October 21, 1939

We have to report herewith some of the results of our karyological studies during 1938. The technique used was the same as before.

Plants used were all raised from seeds derived from botanical gardens in Europe, to the authorities of which the writer wishes to express his cordial thanks.

### The Mode of Cell Division

The plants described here all show the furrowing process in the mode of partition wall formation of the pollen mother cells (cf. Sugiura) (1936b).

### Description

#### Campanulaceae

*Lobelia triquetra*. The genus *Lobelia* has been karyologically studied by several cytologists as shown in my previous paper. The meiotic numbers of chromosomes already found were 7, 8, 9 and 21, among which 7 is the most common. So most investigators have thought that 7 was the basic number in *Lobelia*, but the writer now considers that 8 is the basic number in this genus, although plants having 8 are rare, and that 7 is derived from 8.

This idea is well supported by the fact that there are two meiotic chromosome numbers 7 and 8 in one P.M.C. of *L. inflata* and the meiotic chromosomes of 7 have a large fused chromosome which was described in our previous paper.

The different numbers of chromosomes in the P.M.Cs. of one single flower were also stated by H. Emme and H. Schepeljeva for *Linum crepitans*.

The view that 8 is the basic number in this family will also be found in Wanscher's paper (1934).

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\* The expenses of the present work were partly defrayed out of a grant from the Department of Education.

**Cucurbitaceae**

*Sechium edule*. This is native to tropical America. It belongs to Sicyoideae and has 12 meiotic chromosomes. This number is also found in *Sicyos*, another genus of Sicyoideae. Besides there are 10, 11 and 13 meiotic numbers of chromosomes in other genera of the family, all of them being probably derived from 12, and the theoretical basic number is 4.

**Acanthaceae**

*Jacobinia carnea*. The pollen mother cells are very large like those of others in the family. The meiotic chromosome number is 16. Hence the basic chromosome number in *Jacobinia* is 8 and the theoretical basic number is 4.

**Gesneriaceae**

*Didymocarpus lavandulacea*. The species belonging to Didymocarpaceae has 18 meiotic chromosomes.

The meiotic chromosomes, being spherical, are almost equal in size to those of the Streptocarpaceae. The basic number is 9.

*Streptocarpus Saundersii*. This has 16 meiotic chromosome number, which was also found earlier in other species of the *Streptocarpus* by the present writer. The basic number may be 8. Other species previously studied in the family were *Sinningia* (14), *Corytholoma* (14), *Naegelia* (12) and *Saintpaulia* (14). Thus we now know that 6, 7, 8 and 9 are the basic numbers in the family, the former numbers 6 and 7, being found in Gesnerioideae, while 8 and 9 are found in Cyrtandroideae. But *Saintpaulia*, belonging to Cyrtandroideae, has only 14 meiotic chromosomes, while *Ramondia*, being in the same Ramondieae as the former, has 18 and 36 (Glišić 1924).

**Scrophulariaceae**

*Mimulus cardinalis*, *M. betonicifolia*. Brozek (1930, 31, 32) found 16 somatic chromosomes in *M. cardinalis* and ca 64 in *M. quinquevulnerus*, *M. tigrinus* and *M. tigrinoides*. The writer has found 8 meiotic chromosomes in *M. cardinalis* and 16 in *M. betonicifolia*. The basic number in the genus is 8.

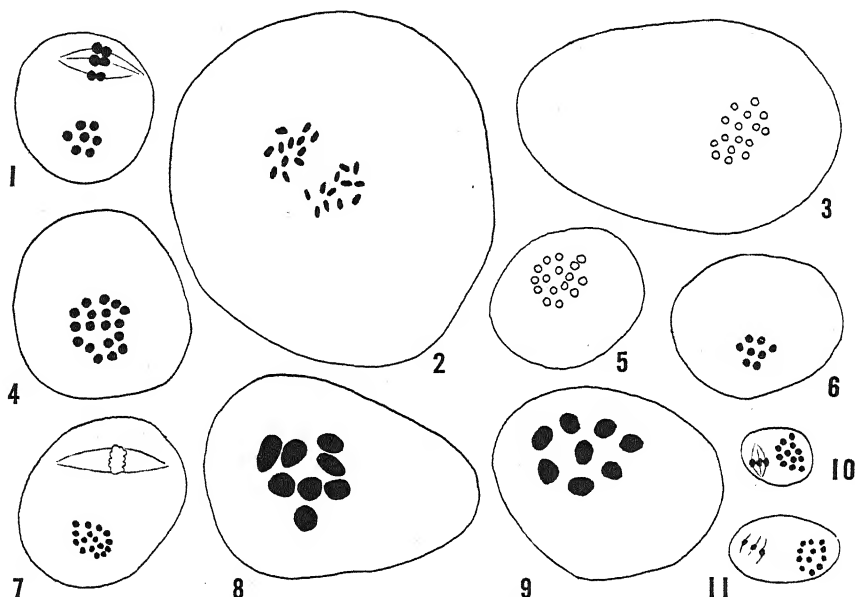
**Borraginaceae**

*Anchusa capensis*, *A. officinalis*. Formerly the writer counted 32 somatic chromosomes in the root-tip cells and later found 16 meiotic chromosomes in *A. italica*. In 1930 Strey found 8 meiotic chromosomes in *A. myosotidiflora* and *A. officinalis*.

The pollen mother cells are very much larger as are also the chromosomes. The theoretical basic chromosome number is 4.

*Cynoglossum creticum*, *C. linifolium* (= *Omphaloides linifolia*). According to our studies the number of n-chromosomes is 12 each.

Strey, however, found 14 meiotic chromosomes in *C. linifolium*. *Anchusa* is a much larger type than *Cynoglossum*, and the pollen mother cells of the former are also much larger than those of the latter.



Figs. 1-11.  $\times 3330$ . 1, *Lobelia triquetra*. IIM. 2, *Sechium edule*. IIA. 3, *Jacobinia carnea*. IIM. 4, *Didymocarpus lavandulacea*. IM. 5, *Streptocarpus Saundersii*. IIA. 6, *Mimulus cardinalis*. IIM. 7, *M. betonicifolia*. IIM. 8, *Anchusa capensis*. IM. 9, *A. officinalis*. IM. 10, *Cynoglossum linifolium*. IIM. 11, *C. creticum*. IIM.

The pollen mother cells of the latter are so exceedingly small that we know very little about them except for *Cuphea petiolata* and *Corrigiola littoralis*. The pollen mother cells of *Cynoglossum linifolium* are only  $5 \times 4 \mu$ . As far as we know, the chromosome numbers found in this family are 8, 12 and 16. Therefore we propose 4 for the theoretical basic number of this family.

#### Apocynaceae

*Amsonia Tabernaemontana*. The pollen mother cells are very large (about  $25 \mu$  in diameter like those of other plants studied in this family). It has 16 meiotic chromosomes. Schürhoff and Müller (1937) also counted the same.

#### Zygophyllaceae

*Tribulus terrester*. Of the chromosome numbers in this family there has been no record yet; the writer has now counted 12. We also know that Erythroxylaceae which is near to Zygophyllaceae ac-

cording to Hoeffgen's serodiagnostic pedigree, also has 12 meiotic chromosomes.

### Linaceae

*Linum africanum*. No previous counts of the chromosome numbers of plants in the section *Linastrum* are available, but we have found 16 meiotic chromosomes in the above plant belonging to the same section. The pollen mother cells are very large in comparison with external appearances. The genus *Linum* contains about 90 species, about 28 of which have been studied karyologically. Four chromosome numbers are known; 8, 9, 15 and 16.

Table 1. Chromosome numbers in *Linum*

Plant name	n	Investigators
<i>Linum alpinum</i> L.	9	de Vilmorin and Simonet 1927b
<i>L.</i> " Jacqu.	18	Kikuchi 1926, 29
<i>L. altaicum</i>	9	Martzenitzina 1927, Kikuchi 1929
<i>L. angustifolium</i>	15 (16)	de Simonet 1929
	15	Reynders 1926, de Vilmorin and Simonet 1927b, Kikuchi 1929
	+16	Martzenitzina
<i>L. austriacum</i>	9	Kikuchi 1926, 29, Fischach 1933
	+9	Martzenitzina 1927
	9, 27/2, (10?)	Freiburg 1933
<i>L. catharticum</i>	8	de Vilmorin and Simonet 1927b
<i>L.</i> " var.	+>57/2 (!)	Martzenitzina 1927
<i>L. extraaxillare</i>	9	Kikuchi 1929
<i>L. grandiflorum</i>	9	Kikuchi 1926
	8	de Vilmorin and Simonet 1927b, Nikolajewa 1927
	+8	Martzenitzina 1927, Sugiura 1936
<i>L. hirsutum</i>	8	de Vilmorin and Simonet 1927b, Fischbach 1933
<i>L. hologynum</i>	9	Kikuchi 1929
<i>L. Lewistii</i>	9	" 1926, 29
<i>L. Muelleri</i>	9	" 1929
<i>L. narbonense</i>	9	" 1929
<i>L. perenne</i>	9	Kikuchi 1926, 29, Nikolajewa 1927, de Vilmorin and Simonet 1927, Martzenitzina 1927, Inouye 1929
<i>L. rigidum</i>	15	Dillman 1933
<i>L. sibiricum</i>	9	Kikuchi 1926, 29
<i>L. sulcatum</i>	15	Dillman 1933
<i>L. usitatissimum</i>	15	Kikuchi 1926, 29, Simonet 1929, Dillman 1933, Kappert 1933, 35
	+15	Reynders 1926, Nikolajewa 1927
	+16	Martzenitzina 1927, Inouye 1929
		Emme and Schepeljeva 1927
<i>L. punctatum</i>	+9	Martzenitzina 1927
<i>L. capitatum</i>	12	Kikuchi 1926
<i>L. flavum</i>	+15 (16)	Martzenitzina 1927
	15	Nikolajewa 1927, Kikuchi 1929
<i>L. tenuifolium</i>	9	de Vilmorin and Simonet 1927
	+9	Martzenitzina 1927
<i>L. corymbiferum</i>	15	Kikuchi 1926
	+9	Martzenitzina 1927
<i>L. maritimum</i>	10	de Vilmorin and Simonet 1927
<i>L. campanulatum</i>	14	de Vilmorin and Simonet 1927
<i>L. salsoides</i>	9	de Vilmorin and Simonet 1927
<i>L. strictum</i>	9	de Vilmorin and Simonet 1927
<i>L. nervosum</i>	15	de Vilmorin and Simonet 1927



It has been found that there are two chromosome numbers in one species.

For example, in *L. grandiflorum*, while Kikuchi counted 9 meiotic chromosomes, Vilmorin and Simonet (1927), Kikolajewa (1927), Martzenitzina (1927) and the present writer counted only 8. Reynders ('26) found for *L. angustifolium*  $2n = 30$ , de Vilmorin with Simonet ('27)  $n = 15$ , whereas Martzenitzina ('27) stated  $2n$  to be 32. Nikolajewa found for *L. usitatissimum*  $2n = 30$ , de Vilmorin with Simonet ('27) and Kikuchi ('26) found  $n = 15$ , but Emme with Schepeljeva, having carried out an extensive study of the chromosomes of a large number of different varieties of *L. usitatissimum*, found in general  $2n = 32$ . Martzenitzina ('27) also found  $n = 32$ . In our opinion 3 being the theoretical basic number, 9 and 15 are derived from it and in this way 8 is probably derived from 9 by the fusion of two of them (cf. Sugiura 1939b).

We do not think 8 and 16 are typical. The chromosome numbers which have been counted to date show that  $n = 9$  and  $n = 15$  plants are much more numerous than  $n = 8$  ones (see Table 1). If we put numbers of  $n = 9$  plants and of  $n = 15$  ones together (9 and 15 both being derived from the theoretical basic number in the genus) it will be seen that our conclusion will probably be endorsed as correct. If the theoretical basic number 3 is applied to F. Hoeffgen's sero-diagnostic phylogenetic tree (cf. Strasburger's 'Lehrbuch' 1923), it will be easily seen that the sero-diagnostic result and our karyological result exactly correspond as shown in the diagram 1.

#### Portulacaceae

*Calandrinia caulescens*. In the previous paper we presented some species of *Calandrinia*. We now introduce another species. This has 24 meiotic chromosomes which are somewhat larger than those of *C. procumbens*.

#### Aizoaceae

The chromosome number in the family had been quite unknown until we counted in 1931 the meiotic number of chromosomes in

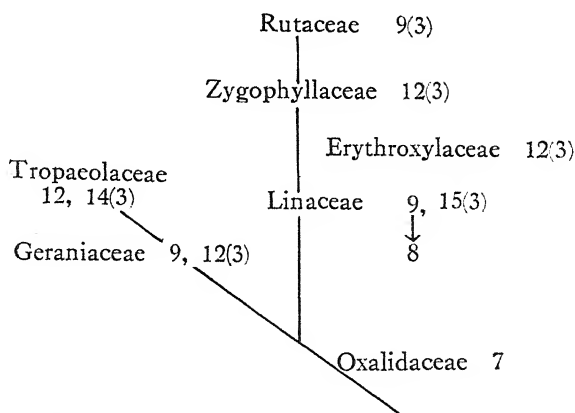
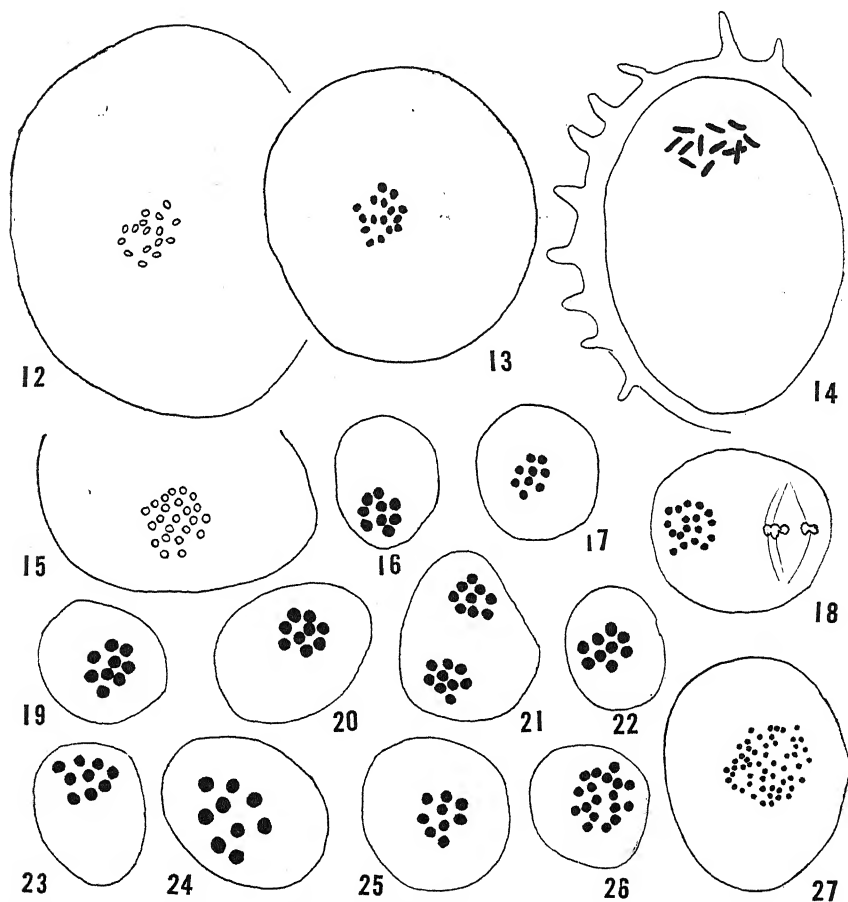


Diagram 1. Drawn in combination of our results and of F. Hoeffgen's sero-diagnostic phylogenetic tree.

*Mesembryanthemum tricolor* to be 9. Since then we have counted the meiotic numbers of chromosomes in the following 11 species of the genus and found 9 in 8 species and 18 in 2 species belonging to the genus *Drosanthemum*. Though *Glottiphyllum* presents rather

		n		n
<i>M. angustum</i>	( <i>Glottiphyllum</i> )	9	<i>M. muricatum</i>	9
<i>M. bigibberatum</i>	(,,)	9	<i>M. obliquum</i>	( <i>Glottiphyllum</i> ) 9
<i>M. candens</i>	( <i>Drosanthemum</i> )	18	<i>M. pomeridianum</i>	( <i>Carpanthea</i> ) 9
<i>M. conspicuum</i>		9	<i>M. uncatum</i>	( <i>Glottiphyllum</i> ) 9
<i>M. difforme</i>	( <i>Glottiphyllum</i> )	9	<i>M. vespertinum</i>	( <i>Drosanthemum</i> ) 18
<i>M. fulgidum</i>		9		



Figs. 12-27.  $\times 3330$ . 12, *Amsonia Tabernaemontana*. IM. 13, *Linum africanum*. IM. 14, *Tribulus terrester*. Pollen. 15, *Calandrinia caulescens*. IM. 16, *Mesembryanthemum angustum*. IA. 17, *M. bigibberatum*. IA. 18, *M. candens*. IIM. 19, *M. conspicuum*. IM. 20, *M. difforme*. IM. 21, *M. fulgidum*. IIM. 22, *M. muricatum*. IM. 23, *M. obliquum*. IIM. 24, *M. pomeridianum*. IM. 25, *M. uncatum*. IM. 26, *M. vespertinum*. IM. 27, *Rivina tinctoria*. IM.

monstrous and somewhat polyploid appearance, it has only 9 meiotic chromosomes. The number 9 is the basic number in the genus *Mesembryanthemum* (Old classification).

The pollen mother cells and the chromosomes in *Carpanthea pomeridiana* are larger than those of other genera. The pollen mother cells and chromosomes in *Delosperma* are much smaller than those of *Mesembryanthemum* and *Glottiphyllum*. The facts enumerated above show that Brown's new classification of *Mesembryanthemum* is also more or less supported by the results of our karyological studies.

### Phytolaccaceae

*Rivina tinctoria*. The chromosome numbers of *Rivina* had not been found until our study was published in 1936. Formerly the

Table 2

Plants investigated	n	IM( $\mu$ )	IA( $\mu$ )	IIM( $\mu$ )	IIA( $\mu$ )	Figs.
Campanulaceae						
<i>Lobelia triquetra</i>	7			0.75		1
Curcubitaceae						
<i>Sechium edule</i>	12				0.87×0.45	2
Acanthaceae						
<i>Jacobinia carnea</i>	16			0.625		3
Gesneriaceae						
<i>Didymocarpus lavandulacea</i>	18	0.75				4
<i>Streptocarpus Saundersii</i>	16				0.625	5
Scrophulariaceae						
<i>Mimulus cardinalis</i>	8			0.625		6
<i>M. betonicifolia</i>	16			0.5		7
Boraginaceae						
<i>Anchusa capensis</i>	8	2.0				8
<i>A. officinalis</i>	8	1.5				9
<i>Cynoglossum linifolium</i>	12			0.5		10
<i>C. creticum</i>	12			0.5		11
Apocynaceae						
<i>Amsonia Tabernaemontana</i>	16	0.5×0.625				12
Linaceae						
<i>Linum africanum</i>	16	0.5×0.625				13
Zygophyllaceae						
<i>Tribulus terrester</i>	12					14
Portulacaceae						
<i>Calandrinia caulescens</i>	24	0.625				15
Aizoaceae						
<i>Mesembryanthemum angustum</i>	9		0.875			16
<i>M. bigibberatum</i>	9		0.65			17
<i>M. candens</i>	18			0.625		18
<i>M. conspicuum</i>	9	1.0				19
<i>M. difforme</i>	9	1.0				20
<i>M. fulgidum</i>	9			0.75		21
<i>M. muricatum</i>	9	1.0				22
<i>M. obliquum</i>	9			1.0		23
<i>M. pomeridianum</i>	9					24
<i>M. uncatum</i>	9	0.875				25
<i>M. vespertinum</i>	18	0.75				26
Phytolaccaceae						
<i>Rivina tinctoria</i>	54	0.375				27

writer counted 54 meiotic chromosomes in *Rivina humilis*. The same number was also counted by Joshia (1936).

Now we have also found 54 meiotic chromosomes in *R. tinctoria*.

Sometimes we have observed the association of chromosomes (two groups of three). Hence the basic number of *Rivina* is probably 18.

The results of the present study are summarized in Table 2.

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## Über die Wirkung verschiedener Pufferlösungen auf die *Spirogyra*-Zellen

Von

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Literatur und Wissenschaft

(Mit 8 Abbildungen)

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Gelegentlich der Kultur- und Vitalfärbungsversuche an *Spirogyra*-Zellen fiel uns eine merkwürdige Tatsache auf, daß verschiedenartige Salze, wie sie auf gebräuchliche Nährflüssigkeiten sowie Pufferlösungen Anwendung finden, wenn jedes einzeln wirkt, auf die *Spirogyra*-Protoplasten nicht geringen schädigenden Einfluß ausüben. Es hat sich herausgestellt, daß diese Wirkung der Salze mit demselben Kation (K) bei demselben pH jenach der Anionenart in verschiedenen Maßen zur Entfaltung kommt. Es handelt sich also hierbei um Anionenwirkung. Im Gegensatz zur Kationenwirkung liegt uns bisher noch keine ausführliche diesbezügliche Angabe bei *Spirogyra* vor. Es scheint sich der Mühe zu lohnen, die Anionenwirkung bei diesem Material näher zu untersuchen.

### Methodisches

Die zur Anwendung kommenden *Spirogyren* wurden im botanischen Garten unserer Universität gesammelt. Ihre spezifischen Namen waren unbestimmt. Sie gehören wenigstens zu 3 Arten von verschiedenen Zellgrößen. In den meisten Versuchen kamen nur die größtzelligen in Betracht, denen Gallertscheide fehlt (vgl. YAMAHA u. ARAKI 1939).

Geprüft wurden außer verschiedenen Sulfaten, folgende Arten der Pufferlösungen verschiedener Konzentration (0,001, 0,005, 0,01 und 0,05 GM) :

1.  $\text{KH}_2\text{PO}_4 + \text{K}_2\text{HPO}_4$  (pH 5,0 6,0 7,0 8,0 9,0).
2. Na- bzw. K-Azetat + Essigsäure (pH 4,0 5,0 6,0 7,0).
3. Mono K-Citrat + KOH (pH 4,0 5,0 6,0).
4. Mono K-Phthalat + KOH (pH 4,0 5,0 6,0).

Zur Herstellung der Lösungen wurde das aus Glas umdestillierte Wasser benutzt, in dem *Spirogyren* für gewöhnlich wenigstens 24 Stunden gesund leben. Aus dem Teichwasser wurden *Spirogyra*-

Fäden 5–15 Minuten in das umdestillierten Wasser eingetaucht. Nach flüchtigem nochmaligen Spülen mit umdestilliertem Wasser ließen wir sie verschieden lang in Pufferlösungen verweilen. Mikroskopische Beobachtung erfolgte unter Anwendung von Kompensationsokular  $20\times$  oder  $30\times$ , und apochr. Objektiv  $20\times$ ,  $40\times$  oder achr. Objektiv  $90\times$  (ZEISS). *Spirogyra*-Fäden wurden immer mit einer zum Haken gebogenen Glasnadel aufgefangen.

### Verschiedene Arten der Strukturanomalien der *Spirogyra*-Protoplasten

Wir möchten nun die Beschreibung verschiedener Strukturanomalien vorausschicken, wie sie *Spirogyra*-Zellen in verschiedenen Salzlösungen aufweisen.

1. Als das erste Stadium verschiedener Strukturanomalien der *Spirogyra*-Zellen tritt häufig die Störung der spiraligen Anordnung der Chloroplasten auf (Abkürzung in der Tabelle—S).

2. Chloroplastentrennung (Abkürzung—T): Die Chloroplastenbänder lösen sich teilweise von dem Wandbelege des Zytoplasmas ab. Dabei wird das wandständige Zytoplasma zwischen dem Chloroplastenband und der Zellwand zu dünnen Strängen, breiten Brücken oder unregelmäßigen Schichten ausgezogen (vgl. SAKAMURA 1933, S. 302). Chloroplastentrennung tritt in der Regel als Vorläufer der Chloroplastenkontraktion in die Erscheinung.

3. Chloroplastenkontraktion (Abkürzung—K, vgl. GICKLHORN 1933):

Chloroplastenbänder werden unter Beibehaltung spiraliger Anordnung ins Zellinnere oder an eine Seite der Zelle zusammengezogen. Dabei kommen im Allgemeinen Plasmafäden zum Vorschein, die sich zwischen dem Wandbelege und den Chloroplasten spannen (Abb. 2).

4. Chloroplastendisintegration (Abkürzung—D): Das erste Anzeichen für Chloroplastendisintegration besteht in der Oberflächenveränderung der Chloroplasten und zwar in der Regel in der Oberflächenverminderung (Abb. 3) und seltener Oberflächenvergrößerung. In normalem Zustand sind Chloroplastenränder gelappt bzw. gewellt und die Innenseite der Chloroplastenbänder ragt kielartig in den Zellsaftraum hinein (Abb. 1) (vgl. SAKAMURA 1933, S. 291). Die Oberflächenverminderung der Chloroplasten kommt nun darin zum Ausdruck, daß die letzteren ganzrandig werden und die kielartigen Vorsprünge derselben verschwinden. Die Oberfläche der Chloroplasten kann dadurch vergrößert werden, daß ihre Ränder zackig werden und die Chloroplastenbänder seitlich miteinander anastomosieren, was bei niederem pH der Lösungen häufig der Fall ist.

Das nächstfolgende Stadium der Chloroplastendisintegration stellt entweder der tropfige Zerfall (Abb. 4) oder die Quellung (ohne Fragmentation) der Chloroplasten dar (vgl. SCHINDLER 1938, S. 192) (Abb. 5,6). Der tropfige Zerfall der Chloroplasten pflegt ohne Störung der spiraligen Anordnung derselben stattzufinden. Bei den alkalischen Lösungen wird die Quellung immer von der Vakuolisierung begleitet (Abb. 7, 8).

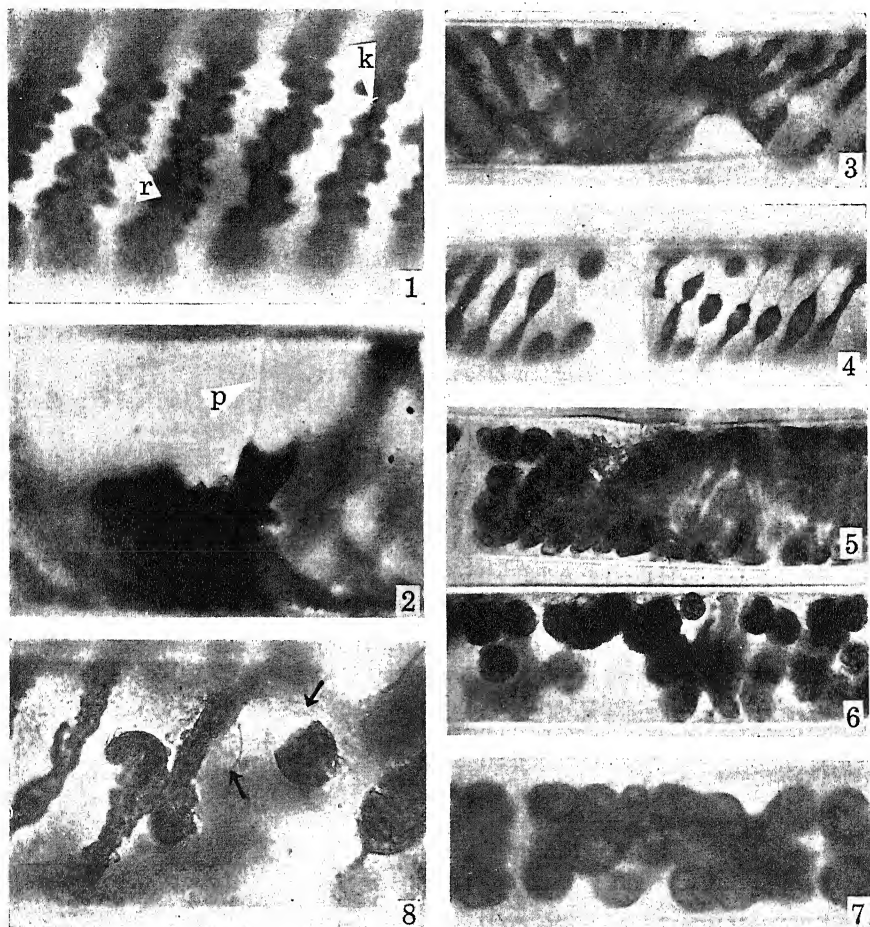


Abb. 1-8. *Spirogyra* sp. 1, Normale Chloroplastenbänder mit Randlappen (r) und kielartigen Vorsprüngen (k). 2, Chloroplastenkontraktion in 0,01 GM Phthalat-Puffer (pH 8,8) (60 Minuten). p, Plasmafaden gespannt zwischen dem Chloroplastenband und der Zellwand. 3, Früheres Stadium der Chloroplastendisintegration in 0,02 GM Zitratpuffer (pH 4,5) (30 Minuten). 4, Tropfiger Zerfall der Chloroplasten in 0,02 GM Zitratpuffer (pH 4,5) (60 Minuten). 5, Chloroplastendisintegration in 0,01 GM Phosphatpuffer (pH 8,0) (4 Stunden). 6, Tropfiger Zerfall der Chloroplasten unter Quellung in 0,01 GM Phosphatpuffer (pH 8,0) (4 Stunden). 7, Vakuolisierung gequollener Chloroplasten in 0,01 GM Phosphatpuffer (pH 7,8) (60 Minuten). 8, Blasenförmige Vakuolisierung der Chloroplasten in 0,02 GM Zitratpuffer (pH 4,6) (60 Minuten). Vergrößerung: 1. 540 $\times$ , 2, 8. 450 $\times$ , 3-7. 225 $\times$ .

Das normale Zytoplasma zeigt Mikrosomen, die BROWNScher Bewegung oder lebhafter Strömung unterworfen sind. Der lebende Zellkern besitzt eine längliche Gestalt. Sein verschwommener Umriß erscheint in geschädigtem Zustand deutlich. Die Form des gestorbenen Kerns ist meistens rundlich.

### Versuche und Besprechung deren Ergebnisse

Die Versuchsergebnisse sollen unten übersichtlichkeithalber tabellarisch zusammengestellt werden. In den Tabellen werden verschiedene Grade der Strukturanomalien mit dem Zeichen + bemerkt. Größere Zahl von + zeigt erhöhte Wirkung betreffender Lösung. So z.B.:

- bedeutet .... Sämtliche Zellen normal.
- ± .... Einige Zellen mit ungeordneten Chloroplasten.
- + .... Ungeordnete Chloroplasten, Chloroplastentrennung oder -disintegration vereinzelt bemerkbar.
- ++ .... Chloroplastendisintegration oder sonstige Anomalien bei 1/5 bis 1/3 von untersuchten Zellen bemerkbar.
- +++ .... Chloroplastendisintegration ungefähr bei der Hälfte der Zellen bemerkbar.
- ++++ .... Chloroplastendisintegration bei den meisten Zellen.
- × .... Zytoplasma bei einigen Fäden koaguliert u. fixiert.
- ×× .... Zytoplasma bei noch mehr Zellen koaguliert u. fixiert.
- ××× .... Zytoplasma bei der Hälfte der Zellen koaguliert u. fixiert.
- ×××× .... Zytoplasma koaguliert, die meisten Zellen im fixierten Zustand tot.

Folgenden Abkürzungen entsprechen in den Tabellen die verschiedenen Arten beobachteter Strukturanomalien:

- S .... Störung der spiraligen Anordnung der Chloroplasten.
- T .... Chloroplastentrennung.
- Dg.... Chloroplasten ganzrandig.
- Dq.... wie oben, aber mit Andeutung an die Quellung
- Dz.... Chloroplastenränder zackig.
- Z .... Tropfiger Zerfall der Chloroplasten.
- Q .... Quellung der Chloroplasten.
- V .... Vakuolisierung der gequollenen Chloroplasten.
- Dk.... Chloroplastendisintegration unter Kontraktion.
- K .... Chloroplastenkontraktion.

Tabelle 1. K-Phosphat (0,005 GM):

Min.	pH	5,0	6,0	7,0	7,4	8,0	9,0
30		—	—	—	+	+	++++
60		± T	± T	+	++	++ Dq, Q, V	++++ Dq, Q, V



Tabelle 2. K-Phosphat (0,01 GM):

Min.	pH	5,0	6,0	7,0	8,0	9,0
20		—	+	T, Dg, Dq	T, Dg, Dq, Q	T, Dg, Dq, Q
30		+	+	++		
60		+	++	++++ T, Dq, Q, V	++++ Q, V	++++ Q, V
180		Dg, Dq	++ Dg, Dq	++++ Dq, Q, V		

Tabelle 3. K-Phosphat (0,05 GM):

Min.	pH	4,7	5,0	6,0	7,0	7,4	8,0	8,6	9,0
10		+	+ Dq	++	± S, T		+ K, Dk	±	
20		++	++	+++	++				
30		+++	++++ Dq, K, Dk	++++	++		+	+	
60			++++ Dg, Dq	++++ Dg, Dq	++	+ Dg, Dz	+ Dg, Dz, Z		+ Dg, Dz, Z
120			++++	++++	++ Dg, Dq		+ Dq, Q	+ T, Dg, Dq	
200			++++	++++	++++	++++ Dq, Q	++++ Dq, Q	++++ Dq, Q	++++ Dq, Q

Tabelle 4. Na-Azetat (0,001 GM):

Min.	pH	4,0	5,0	6,0	7,0
10		+	+	±	—
30		++	Dq, Dk	+ T, K	—
60		++++	++	±	—

Na-Azetat (0,005 GM):

Min.	pH	4,0	5,0	6,0
30		++++ Dq	++++ K, Dq	+

Tabelle 5. Na-Azetat (0,01 GM):

Min.	pH	4,0	5,0	6,0	7,0
10		++ T, K, Dq	++ Dk	± S	
20		+++ T, K, Z, Dq	+++ Z, Dq, Dk	+	
30		xxxx	xxxx	+ T	
60		xxxx	xxxx	+ T	
90		xxxx	xxxx		++ S, T

Tabelle 6. K-Zitrat (0,001 GM):

Min.	pH	4,0	5,0	6,0
10		± S, T	+ T, Dg	—
30		++ T, Dk	++ T, Dg	—
60		+++ T, K, DK	++ T, Dk, Dq	—

Tabelle 7. K-Zitrat (0,01 GM):

Min.	pH	4,0	5,0	6,0
10		×××× T, Dg	++ S, K, Z, Dq, Q	+ K, Z, Dq, Q
30		×××× T, Dg, Dq, Dk	++++ Z, Dq, Q	+ S, Dq, Q
60		×××× T, Dg, Dk	++++ Z, Dq, Q	+++ Dq, Q

Tabelle 8. K-Zitrat (0,05 GM):

Min.	pH	4,0	5,0	6,0
10		+ T	—	—
30		××××		
90		××××	++++ S, Z, Q	++++ Dq, Dk

Tabelle 9. K-Zitrat (0,1 GM):

Min.	pH	4,0	5,0	6,0
30		××××	Dz	Dz

Tabelle 10. K-Phthalat (0,001 GM):

Min.	pH	4,0	5,0	6,0
10		++ T, K	± T	—
30		+++ T, Dq, Dk	+ T	± T
60		++++ T, K, Dq	++ S, T, K	± S
120		++++ T, K, Dq	++ T, K	± S

Tabelle 11. K-Phthalat (0,01 GM):

Min.	pH	4,0	5,0	6,0
10		+++ T, K, Dk, Dg, Dq	++ T, Dq	± T
30		××××++++ T, Dq, Dk	++ T, Dq, (Q)	++ T, K, Dq
60		××××	++ T	++ S, K
120			++ T, S	
200			++ T, S	++ T, Dq

Tabelle 12. K-Phthalat (0,05 GM):

Min.	pH	4,0	5,0	6,0
5		× × × × Dz	+ T	+++ T, Dq
15		× × × × Dz, S	++ Dk, Dq	++++ T, Dq, Q
30			++++ Dk, Dq	

Bei der Durchsicht der obenstehenden Tabellen ist vor allem zu ersehen, daß die schädigende Wirkung der Pufferlösungen nicht weniger auf ihrem pH-Wert als auf der Art und der GM-Konzentration derselben abhängig ist. Wie man theoretisch leicht erwarten kann, so tritt die Anionenwirkung jeder Pufferlösung im Vergleich zur H-Ionenwirkung um so deutlicher in den Vordergrund, je höher die GM-Konzentration derselben ist. Demgemäß wird der Wirksamkeitsunterschied einer und derselben Art der Pufferlösungen von verschiedenen pH voneinander bei höheren Konzentrationen minimal (vgl. Tabelle 8, 9, 11 usw.). Besonders auffallend fällt diese Anionenwirkung in die Augen bei Phosphatpufferlösungen, die im weiteren pH-Umfange (pH 5,0–9,0) zur Anwendung kommen können. Phosphationen wirken eigentlich auf Protoplasten anscheinend entquellend.<sup>1)</sup> Dementsprechend wirken sie den quellend wirkenden OH-Ionen gegenüber antagonistisch. Je höher das pH der Pufferlösung wird, umso ausgesprochener tritt die Quellungsanomalien der Chloroplasten ein, die aber bei der höheren Konzentration der Pufferlösung von demselben pH immer seltener zum Vorschein kommt (vgl. Tabelle 1–3). Diese entquellende Wirkung der Anionen kommt auch bei Zitratpufferlösung in einem geringeren Maße zur Geltung (vgl. Tabelle 6–8). Die Azetatpufferlösung zeichnet sich dadurch aus, daß sie bei pH 4,0 und 5,0 auf Protoplasma stark koagulierend wirkt, während bei pH 6,0 die schädigende Wirkung bedeutend geschwächt wird. Diese starke koagulierende Wirkung der Azetatpufferlösung auf Protoplasma erfahren wir auch bei den Pollenmutterzellen (YAMAHA 1938). Es handelt sich hierbei ohne Zweifel eigentlich um H-Ionenwirkung. Azetationen selbst scheint nicht so große Wirksamkeit zuzukommen, wie es gleich unten gezeigt wird (vgl. weiter SAKAMURA 1934). Nach SAKAMURA (a.a.O.) wirken Na-Azetat und K-Azetat quantitativ voneinander etwas verschieden. Es scheint also wünschenswert zu sein, bei unserem Material die Wirkung beider Azetate miteinander zu vergleichen. Aus den Versuchsergebnissen geht aber hervor, daß K-Azetat im Gegensatz zur Angabe SAKA-

1) Nach PAULI gilt bei der Koagulation des natürlichen Ovalbumins folgende Anionenreihe:

SCN < J < Br < NO<sub>3</sub> < Cl < Azetat < Tartrat < Zitrat < PO<sub>4</sub> < SO<sub>4</sub> < F

MURAS, etwas wirksamer ist als Na-Azetat, wie die untenstehende Tabelle veranschaulicht:

Tabelle 13. K-Azetat (0,01 GM):

Min.	pH	3,95-3,90	4,95	5,9
10		×××× Dz		
30		wie oben	××++ Dg, Dz,	++ S, T, K, Dq
60		wie oben	××++ Dg, Dz, Dq	+++ S, T, K, Dq

Na-Azetat (0,01 GM):

Min.	pH	3,8-3,9	4,95	6,0
10		×××× Dz		
30		wie oben	×+++ K, Dk, Dq	++ S, T, K, Dq
60		wie oben	×+++ Dq	++ S, T, K, (Dq)

Um weiterhin über die relative Wirksamkeit verschiedener Anionen klar zu werden, wurden eine besondere Reihe von Versuchen angestellt und zwar mit 0,01 und 0,001 GM Pufferlösungen von pH 4,0, 5,0 und 6,0. Die Versuchsergebnisse werden in Tabellen 14 und 15 zusammengestellt. Daraus ergibt sich ohne weiteres folgende Wirksamkeitsreihe der Anionen:

Zitrat > Phosphat > Azetat > Phthalat

Tabelle 14. Konzentration 0,001 GM, Temperatur 27,5°C

	pH Min.	4,0 60	5,0 60	6,0 60 und 120 Stunden
K-Azetat		++ T, Dk, Dg	+ T, K	— ± S
K-Phthalat		++++ K, Dk, Dg	+ S, T, K	— ± S
K-Phosphat		+++ T, K, Dk, Dq	+ T, K	— ± S
K-Zitrat		++++ K, Dk	++ T, K	+ T ++ T, K, Dq

Tabelle 15. Konzentration 0,01 GM, Temperatur 26°C, 30 Minuten

	pH	4,0	5,0	6,0
K-Azetat		×××× Dg, Dz	×++ T, K, Dz, Dg, (Dq)	+ S, T, K, Dk (Dg)
K-Phthalat		×+++ Dg, Dk	++++ T, Dk, Dq	+ S, T, K
K-Phosphat		×+++ Dk, Dq	++++ K, Dq, Q	++ T, Dq, Q
K-Zitrat		××++ Dk, Dg, Dz	×+++ Dq, (Q)	++++ Dq, Q

Tabelle 15 macht es höchst wahrscheinlich, daß die obenstehende Ionenreihe auch für die quellende Wirkung der Pufferlösungen auf

Chloroplasten zur Geltung kommt. Um zu sehen, welchen Einfluß verschiedene Pufferlösungen auf die Quellung der Gelatinegallerte ausüben, wurden eine Reihe von Modellversuchen angestellt. Die Streifen aus Gelatinegallert ( $4 \times 0,5 \times 0,2$  cm) wurden in 0,1 GM Pufferlösungen von pH 6,0 bei  $22-23^\circ \text{C}$  16 Stunden eingetaucht. Verlängerung der Gallertstreifen durch Quellung betrug wie folgt:

Pufferlösung	Verlängerung der Gelatinestreifen in %
K-Azetat	59,4
K-Phthalat	79,4
K-Phosphat	68,7
K-Zitrat	75,0

Dabei gilt also als Quellungsreihe:

Phthalat > Zitrat > Phosphat > Azetat

Mit Ausnahme von Phthalat gehen somit die Quellung der Gelatinegallerte und Chloroplastendisintegration durch verschiedene Pufferlösungen (wenigstens bei pH 6,0) miteinander genau in Parallele.

Nach BENECKE (1907), SAKAMURA (1922, 1934) und EISELSBERG (1937) entfalten Ca-Ionen bei verschiedenartigen schädigenden Lösungen (angesäuerten Lösungen, NaCl-, KCl-, LiCl- und Na-Azetat-Lösung usw.) auf *Spirogyra*-Zellen entgiftende Wirkung. Untenstehende Tabelle zeigt einwandfrei, wie auch bei unseren Pufferlösungen Ca-Ionen schädliche Wirkung vermindern können.

Tabelle 16. 0,01 GM Pufferlösungen,  $\text{CaCl}_2$ -Zusatz von 0,001 n

	1 Std.	24 Std.
K-Azetat mit Ca (pH 5,85)	—	—
„ ohne Ca (pH 5,8)	+ S, T, K	+++ Z, Dq, Q
K-Phthalat mit Ca (pH 5,9)	± S?	± S
„ ohne Ca (pH 5,9)	+ S, T	++ Dq
K-Phosphat mit Ca (pH 5,8)	—	—
„ ohne Ca (pH 5,8)	+++ Dk, Dq, Z, Q	++++ Dq, Z, Q
K-Zitrat mit Ca (pH 5,7)	++ S, T, Dq, (Q)	++++ Dk, Dq
„ ohne Ca (pH 5,8)	++++ Dq, Q	++++ Dq, Z, Q

In Tabelle 17 können wir die relative Wirksamkeit von KCl,  $\text{CaCl}_2$  und  $\text{MgCl}_2$ , wie sie als Zusatz in den Pufferlösungen vorhanden sind, übersehen. Wir bemerken, daß KCl bei Zusatz von 0,001 n die schädliche Wirkung von 0,01 GM Phosphat-Pufferlösung von pH 5,7 noch merklich verstärkt, während sie durch  $\text{MgCl}_2$  ziemlich und durch  $\text{CaCl}_2$  bedeutend vermindert wird.

Tabelle 17. Phosphat-Puffer 0,01 GM, pH 5,6-5,7, Temperatur 29°C

K-Phosphat ohne Zusatz:	+++ T, Dk, Dq, Z, Q
K-Phosphat mit KCl (0,001 n)	+++ K, Dq, Z, Q <sup>1)</sup>
K-Phosphat mit CaCl <sub>2</sub> (0,002 n)	— S ?
K-Phosphat mit MgCl <sub>2</sub> (0,002 n)	++ Dg, Gq, Z, Q

1) Weiterhin bemerkt man Verschmelzung gequollener Chloroplasten und Vakuolisierung des Zytoplasmas.

Anhangsweise möchten wir hier auch die zufällig beobachtete eigentümliche Wirkung von SO<sub>4</sub>-Ionen auf *Spirogyra*-Protoplasma zusammenfassend berichten. Merkwürdig sei nämlich, daß *Spirogyra*-Zellen zwar mit 0,2 GM MgCl<sub>2</sub> und 0,6 GM Rohrzucker deutlich sich plasmolysieren lassen, aber mit 0,2 GM Na<sub>2</sub>SO<sub>4</sub> und 0,3 GM MgSO<sub>4</sub> nicht plasmolysierbar sind. Die sämtlichen geprüften Sulfate (Na<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub> und CaSO<sub>4</sub>) sind darin einig, daß sie auch in verdünnter Konzentration (hypotonisch) immer deutliche Chloroplastenkontraktion verursachen können. Es hat den Anschein, als ob beide Erscheinungen irgendwie kausal verknüpft sind. Ähnlichen Eindruck haben wir auch bei der Saponinwirkung auf *Spirogyra*-Zellen gemacht (vgl. YAMAHA u. ARAKI 1939). Wenn man die 15-20 Minuten mit 0,3 GM MgSO<sub>4</sub> behandelten *Spirogyra*-Zellen in 0,6 GM Rohrzucker einlegt, so bemerkt man die schwache eckige Plasmolyse, was auf die veränderte Beschaffenheit des Protoplasmas hindeutet.

### Zusammenfassung

1. Verschiedenartige Pufferlösungen von pH 4,0-9,0 bei der Konzentration von 0,001-0,05 GM bedingen verschiedene Struktur-anomalien von Chloroplasten und Zytoplasma.
2. Diese schädliche Wirkung der Salzlösungen, an der neben H- und OH-Ionen auch Anionen wesentlich teilnehmen, läßt sich durch Ca- und Mg-Ionen in verschiedenen Maßen vermindern (Entgiftung).
3. Relative Wirksamkeit der verschiedenen Pufferlösungen von demselben pH geht mit der quellenden Wirkung auf Gelatinegallerte in Parallele. Dabei macht Phthalat-Puffer eine Ausnahme.
4. Es hat sich wenigstens bei Phosphat- sowie Zitrat-Pufferlösungen gezeigt, daß die Anionen in den Pufferlösungen der quellenden Wirkung von OH-Ionen anscheinend entgegenwirken, so daß die höhere Konzentration der Pufferlösung gleichsam wie die Erniedrigung des pH derselben wirkt (vgl. YAMAHA u. ISHII 1932).
5. Sulfat-Ionen machen *Spirogyra*-Zellen schwer plasmolysierbar und bedingen Chloroplastenkontraktion.

Zum Schluß möchten wir der Japanischen Gesellschaft zur Förderung der Zytologie für ihre finanzielle Unterstützung an dieser Untersuchungen unseren aufrichtigsten Dank aussprechen.

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## Chromosome Studies on *Trillium kamschaticum* Pall.

### VIII. On the mitosis-meiosis relationship\*

By

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In a previous paper of this series, No. 5, the senior writer described several abnormalities of chromosome structure and behavior which originated from the subjection of *Trillium* plants to high temperature prior to the meiotic divisions. It was there pointed out that the abnormal divisions thus produced are due to the upsets in timing relationships between the behavior of the kinetochore and that of the chromonema proper.

The same line of research was repeatedly undertaken by the present writers in the following season. This time the plants were placed in a hot chamber in late October and kept at a temperature of 20° C for several weeks until the divisions took place. After such treatments, their PMCs showed various abnormalities in cell division. Some of the irregularities were the same as reported before, but the others were found to be of quite new types. In the present paper, some of them which seem to be directly related to the problem of the mitosis-meiosis relationship will be described and discussed.

### Observations

For a clearer understanding, the chromosome types which are dealt with in the present paper are diagrammatically represented (Fig. 1). Some brief description will be given on them in the order of the grade of alternation in chromosome behavior.

1) **Meiotic.** The normal or standard meiotic chromosomes constitute tetrads which take a cruciferous form. In each dyad four chromonemata (functionally two chromatids) are coiled together in single spirals at early metaphase, which separate however later into two parallel-coiled chromatids. The pair of paired kinetochores (functionally two) is associated together at early metaphase; they separate later two by two orientating themselves along the line of polar attraction.

In contrast with the standard type, the precocious meiotic

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tetrads are precocious in structure in that each of the four chromatids forms an independent spiral at metaphase and the paired kinetochores have already separated into four. The paired chromatids are maintained together by lying within a common matrix.

As previously illustrated, there are distinguishable two types of chromatid association in these tetrads (as well in the standard tetrads), parallel and cross-associations, which occur in the frequency of 1:2 (Matsuura '37).

2) **Mitotic.** This type is characterized by the occurrence of the ten univalents at metaphase instead of the five bivalents. This character originates from the

failure of pairing of homologues. The mode of division is essentially the same as in the root-tip mitosis, the dyad cells each of which contains the ten chromatids being regularly formed (Fig. 2). It is not clear at present whether these dyad cells are functional or not. Here also two chromosome types can be distinguished, "standard" (Fig. 3) and "precocious" (Fig. 7), as in the "meiotic" chromosomes, according to whether the chromatids are associated so closely as to form a single spiral

or are already split to form each an independent coil. It is a remarkable fact that chromosomes presented in Figs. 2-6 are apparently only of minor spirals as in the usual somatic chromosomes, though there is a certain degree of variation in the coil number; the number of coils in chromosome *a* in Fig. 5 was estimated to be about 46, while that in Figs. 3 and 4 to be 35. This makes a marked contrast with

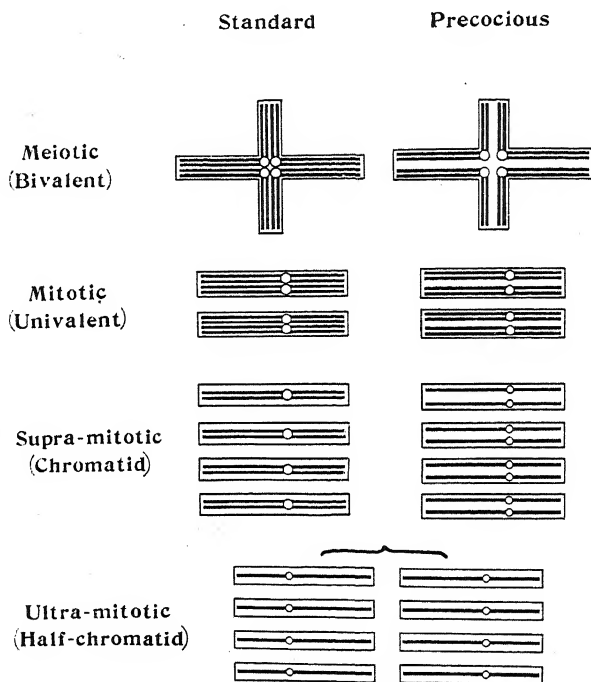


Fig. 1. Diagrammatic representation of various chromosome types. A pair of homologues is shown in each. Black rods represent the chromonemata, circles the kinetochores, and outlines the matrix.

the chromosomes in Figs. 7-9 which are clearly characterized by the spiral-in-spiral structure, for chromosome *a* in these figures shows about 10 major coils. These findings seem to indicate that there is continuous variation in chromosome spiralisisation between the "double-coiled" or spiral-in-spiral structure and the so-called "single-coiled" structure and hence no clear demarcation may be possible between these two types.

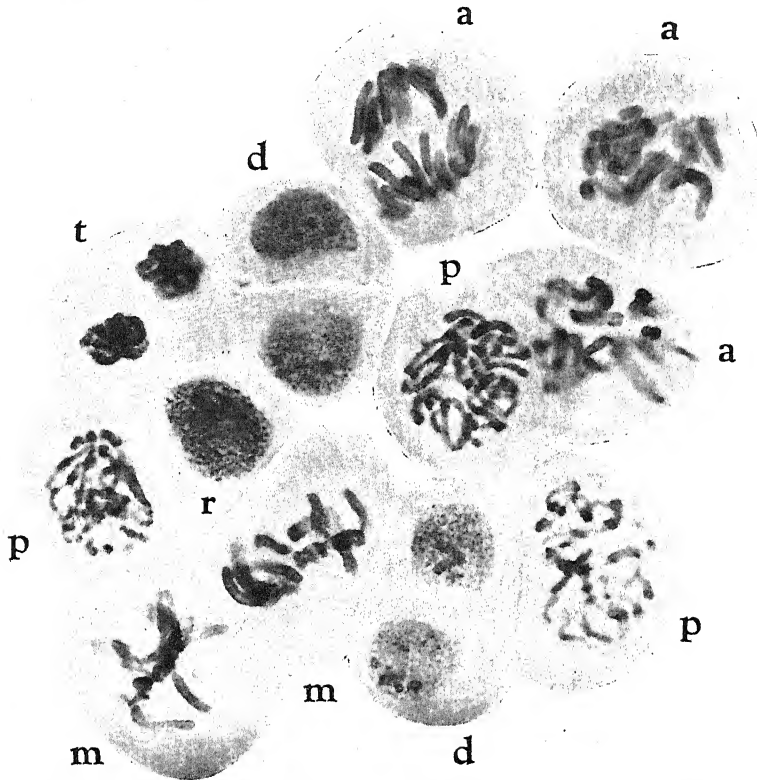


Fig. 2. Twelve PMCs of the "mitotic" chromosomes type. *r* = resting stage, *p* = prophase, *m* = metaphase, *a* = anaphase, *t* = telophase, *d* = dyad formation.  $\times$  ca. 700.

The same situation is also recognizable in the three nuclei, prior to metaphase, of the precocious mitotic type represented in Figs. 10-12. These structural variations may be considered "concordant" with respect to the behavior of the kinetochore and the rest of the chromonema within the mitotic type (*cf.* Matsuura '37).

It is noticed *en passant* that the reversal of coiling direction of the chromonemata occurs in these mitotic chromosomes as indicated by the arrows in Fig. 6. This clearly implies that the change in coiling direction can occur independently of the interstitial chiasmata.

3) **Supra-mitotic.** This type is thus named, because it goes beyond the limit of the usual mitosis with respect to the behavior of the kinetochore. Here the 20 separate chromatids appear at metaphase (Fig. 14), representing a condition of anaphase in the usual mitosis. Such precocious separation of chromatids is already observable in prophase nuclei (Fig. 13). The metaphase of this type is characterized by the non-formation of the regular equator, the chromosomes lying scattered throughout the cell, and accordingly no regular division takes place; either they go at random to the poles (Fig. 15) or they enter into the resting stage without migrating to the poles, forming probably a restitution nucleus (Fig. 16).

So far as the present observations go, the chromosome structure in this type was only that of the so-called double-coiled spiral as in the usual meiotic chromosomes. Chromosome *a* in Fig. 14 showed about 10 major coils throughout its length, but in the chromosomes represented in Fig. 15 the coil number seemed to be much less. Apparently here too is an occurrence of concordant variations in chromosome structure.

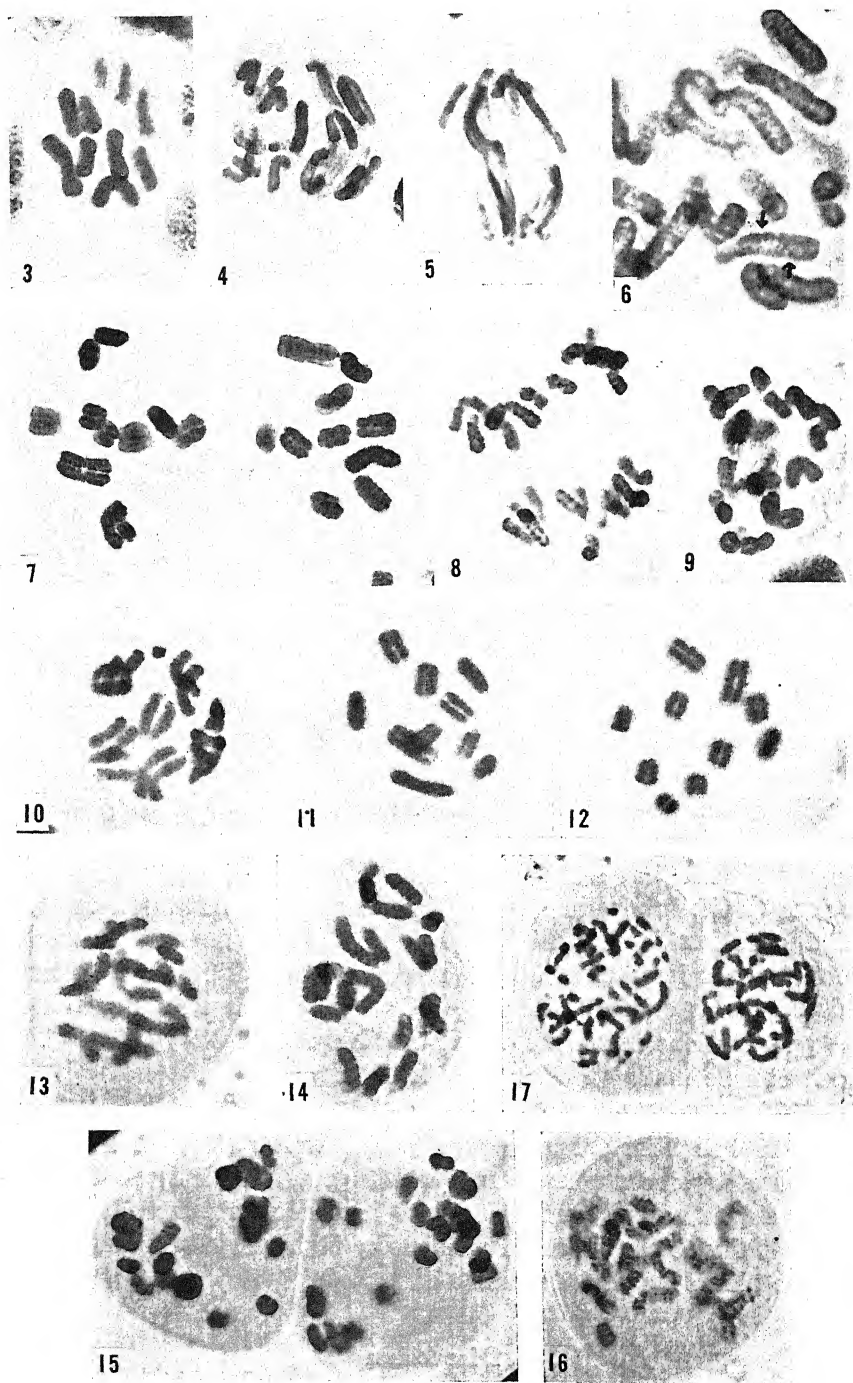
In the present study, no precocious form of this type was found, though it would be expected from the preceding meiotic and mitotic aberrations.

4) **Ultra-mitotic.** Two cells represented in Fig. 17 show that chromosomes are very slender, about half a diameter of the chromatids in Fig. 14, and about 40 in number, though there is certain indication of chromosome fragmentation. Such cells were found relatively rarely in the present experiments, but seem to be enough to lead to the inference that this is a case of the most extreme type of alternation in chromosome formation, viz., a case where the chromosomes have already split into half-chromatids. In this sense this type was called "ultra-mitotic." Since these cells appeared in the preparations mixed with cells of other abnormal types, no statement can be made on their further development.

It is interesting to note here that a case comparable to the present one has been found by the junior writer ('37) in PMCs of *Kinugasa japonica*, when plants which grow wild only in the sub-alpine regions were transplanted to lowlands. Apparently this is similarly due to the effects of abnormally high temperature surrounding the plants on the mechanics of meiosis.

### Discussion

The abnormalities described in the present paper are explicable as the results of various "discordant" behaviors of the kinetochore



Figs. 3-17. Photographs of PMCs of *Trillium* plants which had been kept at 20°C for 25-30 days (from Oct. 26 to Nov. 21-26). 3-6, metaphase (3) and anaphase (4-6) of

and the rest of the chromonema (*cf.* Matsuura '37). A series of events—meiotic, mitotic, supra-mitotic and ultra-mitotic—clearly indicates how the acceleration of the behavior of the kinetochore due to high temperature results in profound alternations in chromosome formation from the normal meiotic chromosomes. In view of this the present findings have important bearing on the interpretation of the mechanism of meiosis.

Some attempts have been hitherto made to homologize the mechanism of meiosis with that of the usual mitosis. They are (1) the precocity hypothesis (Darlington '31, see '37), (2) the unified hypothesis (Huskins '32, '33), and (3) the retardation hypothesis (Stebbins '35, Sax & Sax '35, Beasley '38). The former two assume that the cause of chromosome pairing is the univalency of synapsing chromosomes at the early meiotic prophase.

According to Darlington, meiosis is initiated by a premature prophase contraction which precedes chromosome splitting and this causes the mitotic affinity to be satisfied by the approximation of whole chromosomes instead of split halves as in usual mitoses.

Huskins has made a modification of this hypothesis by saying that meiosis is initiated by retardation or inhibition of the split in the chromonemata during the last premeiotic division rather than by precocious contraction in the meiotic prophase.

The third interpretation is based on evidences showing that the meiotic cycle is a much more leisurely process than the mitotic cycle; in other words, meiosis is associated with a reardation in cell activity rather than with precocity in development. This will cause the uncoiling of the residual spirals of chromonemata at the meiotic prophase, which is held as the essential condition prerequisite to chromosome pairing, gene by gene.

The present investigation supports the third interpretation of meiosis in that, by speeding up the division process, meiosis is convertible into mitosis, not only in the behavior of chromosomes but also in their internal structure. Furthermore the present study clearly demonstrates that the chromosome is at least four-parted already in the meiocyte, as evidenced by the occurrence of the "ultra-mitotic" type of chromosome formation, because in this type, as in the other types, the effect of temperature is initiated at the resting stage, and hence the possibility of exertion of the stimulus at the

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the standard mitotic type; 7-12, "diakinesis" (10-12), metaphase (7) and anaphase (8 and 9), of the precocious mitotic type; 13-16, prophase (13), metaphase (14), anaphase (15) and telophase (16) of the supra-mitotic type; 17, two nuclei of the ultra-mitotic type. All  $\times$  ca. 750, except 6 which was taken at a magnification of  $\times$  ca. 1290, and all reduced to 9/10 in reproduction.

premeiotic division is excluded. This finding contravenes the assumption that pairing occurs because the leptotene threads are unsplit. *A priori* there is little reason for assuming that the last premeiotic division differs from other mitoses; actually several workers, e.g. Nebel and Ruttle '36, Ruttle and Nebel '37, Atwood '37, have offered evidence that the last premeiotic division is normal. It should be also mentioned that Darlington's hypothesis of pairing fails to explain the intimate synapsis of the salivary gland chromosomes which are held to be multivalent in structure (*cf.* Cooper '38).

### Summary

1) Through the subjection of *Trillium* plants to high temperature (20° C) prior to the meiotic divisions, several abnormal types of division were obtained in their PMCs. They were classified into (1) mitotic, (2) supra-mitotic and (3) ultra-mitotic. These are diagrammatically illustrated in Fig. 1.

2) In the mitotic type the 10 separate univalents (dyad chromosomes) appear at metaphase instead of the 5 bivalents.

The supra-mitotic type is characterized by the formation of the 20 separate chromatids, and the ultra-mitotic type by the 40 separate half-chromatids. The occurrence of the last type indicates that the chromosome at meiotic prophase consists of at least four separable chromonemata.

3) In the meiotic and mitotic types, there appeared corresponding "precocious" types, in which the kinetochores have already split further at metaphase and each chromosome consists of two separate chromatids lying parallel within the pellicle and coiling independently.

4) It was demonstrated that meiosis is convertible through acceleration of cell activity into mitosis, not only in the behavior of chromosomes but also in their structure. This implies that meiosis differs in its mechanism from mitosis in the retardation of prophase, not in precocious chromosome development as Darlington assumes.

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## Chromosome Studies on *Trillium kamtschaticum* Pall.

### XII. The mechanism of crossing-over

By

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Of various hypotheses on the cytological mechanism of crossing-over, Darlington's interpretation ('35) seems to be most logically elaborate. He assumes that the cause of the formation of chromonema spirals is in an internal twist due to a rearrangement of the constituent particles, either between molecules, or within molecules. This molecular spiral leads the thread to coil in an internal spiral in the direction opposite to that of the major and minor spirals, and its direction is subject to unitary control in each of the arms of the chromosome; it is reversed at the kinetochore, so that the two arms of a single chromosome always coil in opposite directions. At the end of pachytene each particle undergoes division; the two threads are twisted round one another by relational coiling. Thus the daughter chromatids are relationally coiled, the direction of coiling being the same in each homologue, but just opposite to that in which the chromosomes are relationally coiled. The four chromatids are thus in a state of tension resulting from the conflict of lateral attraction and longitudinal cohesion. This torsional strain may induce a chromatid break in one of the four chromatids; if one breaks, all the stress will fall on the other chromosome, leading to the break of one of its chromatids. This double break will allow the release of the stress by rotation, after which the reunion of the broken ends may take place, thus resulting in crossing-over in the genetical sense and in a chiasma in the cytological sense. Thus the relational coiling in zygotene is supposed to be replaced by chiasmata in diplotene.

This hypothesis seems to be logical, but unfortunately several assumptions on which this hypothesis rests are at variance with the observed cytological facts. Especially the writer must emphasize that evidences were obtained from the present series of investigations on *Trillium kamtschaticum* which are entirely incompatible with these assumptions. First, it is difficult to understand how the broken ends of the two chromatids *always* rejoin in such a way as to give rise to two new chromatids. Darlington merely says ('37,



p. 548): "This rejoining presents no new problem, for as we have seen, ends of different chromosomes, free and unpaired, are capable of rejoining after X-ray breakage." However the X-ray experiments have showed that the reunion of the broken ends takes place at random (*cf.* No. 9 of this series) and hence it is likewise probable that it gives rise to a dikinetid chromatid and a fragment instead of the formation of two new complete chromatids. Such is not involved in crossing over. Secondly, his precocity theory of meiosis has been invalidated by the evidence that leptotene threads are composed of multiple chromonemata as in mitotic chromosomes (*cf.* No. 8 of this series). There is no reason therefore for assuming that the chromosome division takes place at the onset of diplotene and that this causes the upset of the equilibrium which leads to chromatid breakage. Thirdly, recent studies on the direction of chromonema spirals have indicated, in opposition to Darlington's assumptions, that there is no regularity in direction between two homologues nor between the two arms of a chromosome, and changes in direction which are independent of chiasmata in their origin occur in chromosome arms (*cf.* Nos. 2 and 4 of this series).

It should also be mentioned that our critical analysis of the chiasma and experiments on its origin gave evidence clearly contradicting Darlington's idea of chiasma localisation (No. 10 of this series). Furthermore, statistical study on the tetrad configurations and on the behavior of an heteromorphic pair of chromosomes gave results which strongly support the writer's Neo-two-plane theory (No. 7 of this series). Such accumulation of data against the chiasma type theory of Darlington led the writer believe that the "evidences" taken by him and his school as favoring his theory should be critically reinvestigated on a more scientific basis. Studies on this line are now in progress in our Laboratory.

One of the most important aims of this series of investigations has been the elucidation of crossing-over mechanism. The writer has failed to find it out at meiotic prophase and also to get any conclusive evidence that the chiasma is in its origin related to crossing-over. During the course of his study on the chromonema structure of meiotic chromosomes in *Trillium kamtschaticum*, he came to the conclusion that crossing-over *must* take place at the first meiotic metaphase. The data on which this conclusion rests are given in the present paper. The proposal of such an interpretation of crossing-over mechanism may be rather surprising to those who consider that it must take place at meiotic prophase, but scientists must resort to *verae causae* and avoid the formulation of *fictae causae*.

### Terminology

In order to avoid confusion in the terminology, it will be desirable to give here definitions of some spiral forms. The formation of an apparent single spiral by two parallel threads which will be referred to as a "double-thread spiral" is carried out in one of two ways. (1) The spiral can arise by the rotation of both the ends of the threads. If such a spiral is made with a copper wire by wind-

ing them round a rod, keeping the two threads always parallel, and they are pulled out straight without allowing any relative rotation of the ends, it will be found that the copper wires are relationally twisted with one another in the same direction as the spiral was wound, the number of such half-twists being equal to the number of half gyres of the spiral (Fig. 1A). This type of spiral, parallel in three dimensions but twisted in two dimensions, may be termed a "relational double-thread spiral."

(2) The second type of spiral can be formed by winding the wires round a rod without allowing any rotation of the ends. Then in the course of spiralisation a reversed twisting takes place, the number of such half-twists being equal to the number of half-gyres of the spiral. When such spiral is pulled out straight without any relative rotation of the ends, one finds that the two threads are parallel throughout their length, for the twisting produced by spiralisation is here compensated by a twist in the opposite direction at each gyre of the spiral (Fig. 1B). Such a type of spiral, twisted in three dimensions but parallel in two may be termed a "parallel double-thread spiral."

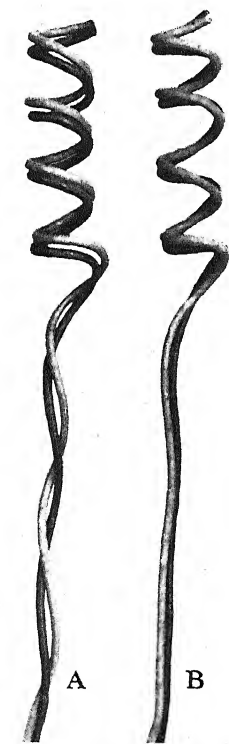


Fig. 1. Models of double-thread spirals, relational (A) and parallel (B).

These double spirals should not be confused with the double-coiled spiral (spiral-within-spiral), because very often the latter is also referred to as a double spiral.

### Observations

What spatial relationship within a spiral system is taken by the paired chromatids at meiotic metaphase is the subject matter of the present observations. Usual cytological techniques proved to give

no satisfactory result, for the two chromatids of early metaphase are tightly appressed into one another by forming minor coils and hence their ease of sticking together by fixatives tends to give them the appearance of being a single-thread spiral. To overcome that tendency the writer's water-pretreatment method (No. 11 of this series) was found to be exceedingly effectual. By this treatment the two chromatids can be traced without much difficulty, especially in permanent smear preparations. In those treated by aceto-carmin after this pretreatment, the minor spirals are well preserved; although such a state will be more natural, it imposes certain difficulty on the observation and it is only by careful focussing that the duality

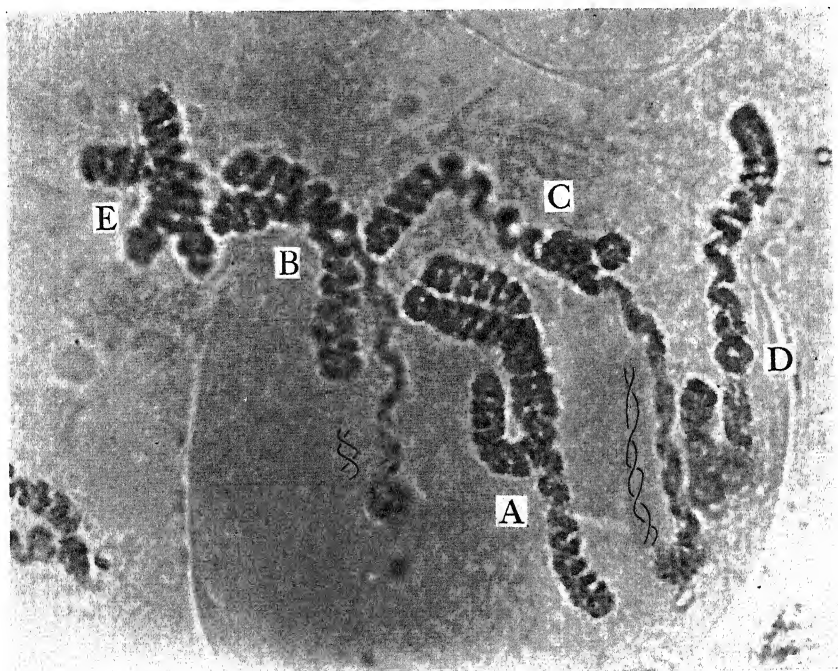
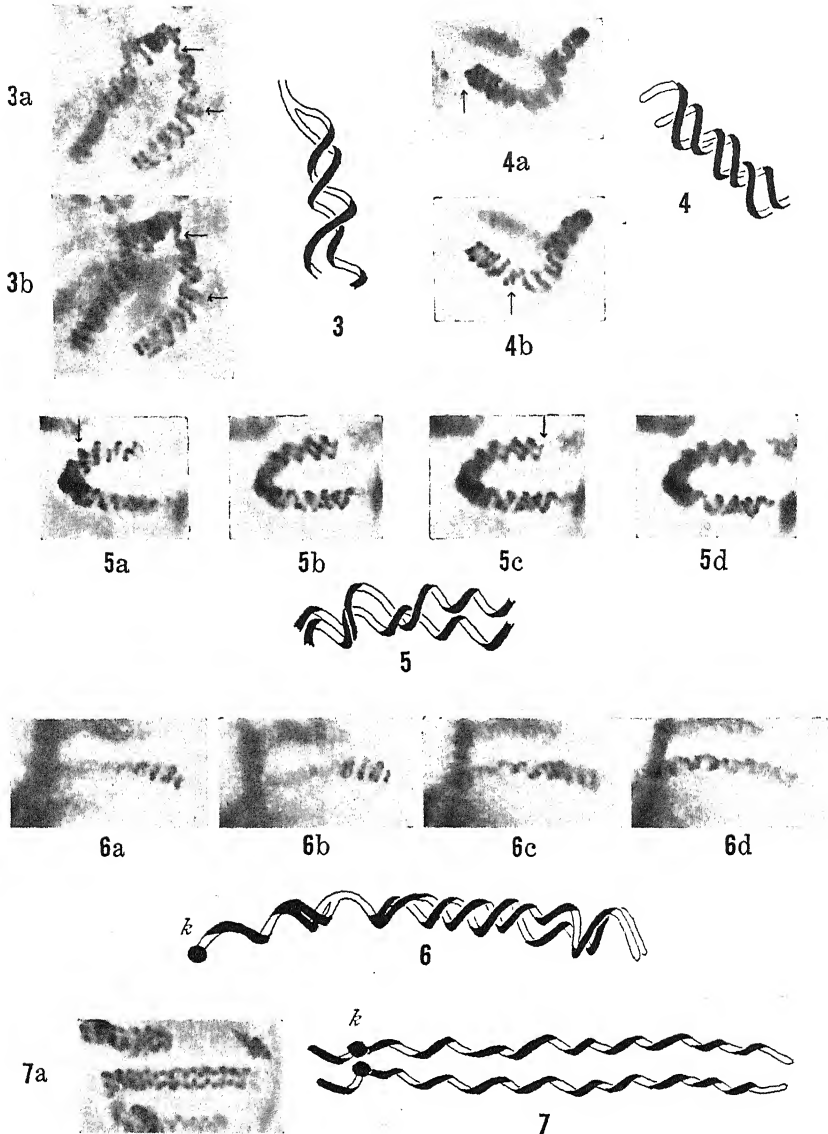
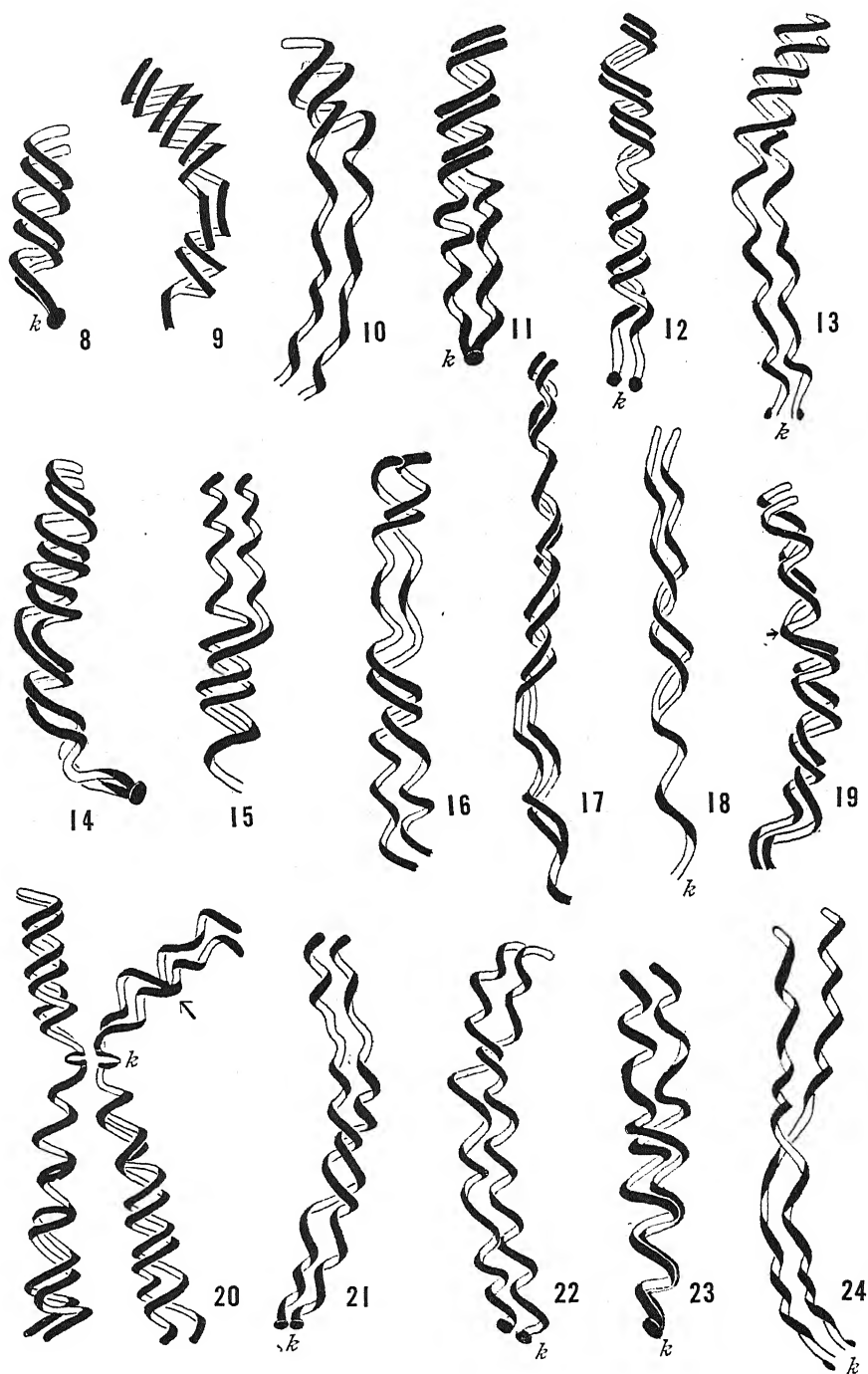


Fig. 2. The five bivalents at early metaphase of a PMC. The microphoto was taken from an aceto-carmin preparation pretreated with water. The pressure which was given on the cover-glass made some portions of chromosomes exceedingly stretched out. The relational relationship of the paired chromatids are revealed in these uncoiled portions, as shown by attended explanatory figures.  $\times$  ca. 1300.

of the spiral can be traced. When however the major coils are artificially uncoiled by gentle pressure on the cover-glass, the two chromatids are clearly discernible (Fig. 2). Most of the present observations were then made on permanent smear preparations, in which the chromonemata are considerably contracted by the fixation so that the minor spiral is obscured, yielding a favorable condition



Figs. 3-24. Illustrating various internal relationships between the paired chromatids. All the figures were made from permanent smear preparations pretreated with water, the fixative being La Cour 2BE and the stain gentian violet. The magnification of the photos is ca. 1300 and that of the sketches ca. 4050. The appended sketches in Figs. 3-6 are those of the regions indicated by the arrows in the corresponding photos. In Figs. 8-24 (excl. Fig. 20), the upper part in each figure is the distal end of the arm. The region marked by *k* is the kinetochore. The identity of these chromosomes are as follows (for denotation, see the previous papers): 3, dl; 4, bs; 5, dl; 6, a; 7, dsl, 8, es; 9, bl; 10, dl; 11, bl; 12, cl; 13, cl; 14, a; 15, cl; 16, cl; 17, a; 18, bl; 19, a; 20, a whole bivalent B; 21, a; 22, bl; 23, a; 24, a.



For explanation, see p. 394.

for disclosing the internal relationship of the two threads. The material was taken from normal sporocytes.

Some earlier workers maintain that the two chromatids of each chromosome are coiled together in single spirals at early metaphase, *e.g.* in *Secale* (Sax '30), *Tradescantia* (Sax and Humphrey '34), *Rhoeo* (Sax '35), *Vicia* (Sax and Sax '35, Sax '36), while others hold that two parallel-coiled chromatids are found at this stage, *e.g.* in *Gasteria* (Taylor '31), *Trillium* (Huskins and Smith '35), *Fritillaria* (Darlington '35). These opposing views seem to the present writer merely to be due to the difference of stages at which the observations were made, for in the present material it is convincingly clear that at early metaphase the two chromatids take the form of single spirals, while at late metaphase they show separate parallel coils.

The nature of this apparent single spiral at early metaphase was found to be that of the relational double-thread spiral system. This is clearly represented in Fig. 2 at the portions of the chromosome arms which were mechanically uncoiled. In order to meet the doubt that such interlacings of the chromatids might be induced by mechanical uncoiling of the major spiral, closer observations were made in uninjured cells of permanent preparations, and it was found that the same structure also holds true, as illustrated by several microphotos, Figs. 3-6.

Then a question can be naturally raised how a relational spiral at early stage is convertible later into a parallel spiral, that is to say, how the process of parallelisation takes place. From numerous observations, the writer finds that there are many intergrading conditions which can be arranged as follows:

- 1) The two chromatids are relationally coiled throughout the entire length of the arm except a certain portion where the direction of coiling is reversed (Figs. 8, 14 and probably 9).

- 2) The proximal part of an arm is parallel and the residual part relational (Figs. 10, 11, 12, 13, 16, and the left short arm of Fig. 20).

- 3) Both the end regions of an arm are parallel, while the intercalary part is still relational (Figs. 19, 21, 22 and probably 6, 15, 17, 18).

- 4) The two chromatids are parallel with the exception of only one intercalary half-twist (Figs. 23 and 24).

- 5) The two spirals are completely parallel throughout their entire lengths (Fig. 6; see also many figures in No. 3 of this series).

Such a series of events must be taken as indicating the direction of parallelisation. As a corollary it is concluded that the process of parallelisation begins first at the kinetochore region, then independently at the distal end of the arm, and that these two processes advance inwards along the arm (see Fig. 25).

During the course of the present observations the following two facts were noticed which may be worthy of mention: (1) that very often the paired chromatids are attached together at an exactly corresponding point so that it is impossible to identify which are the original threads, as shown in the regions indicated by the arrows in Figs. 19 and 20; (2) that the original spiral takes a cylindrical form, while in the parted spiral the dimension along the cleavage surface is unaltered, but when viewed from the plane orthogonal to it the spiral is of about a half width (Figs. 10, 11, 13, 15 etc.). These points are of significance in considering the mechanism of parallelisation (*v. infra*).

The present observations were restricted to the relationship between the two chromatids. The chromatid however is not structurally the final unit; it consists of at least two visible chromonemata. However, since the

chromatid may be taken functionally as an unit, the relationship between these half-chromatid chromonemata has been left untouched in the present study. Furthermore the chromosome arms dealt with in the present study are only those bearing no chiasma. How the chiasma is related to the spiral structure is another problem. Findings on these points will be published in further papers of this series.

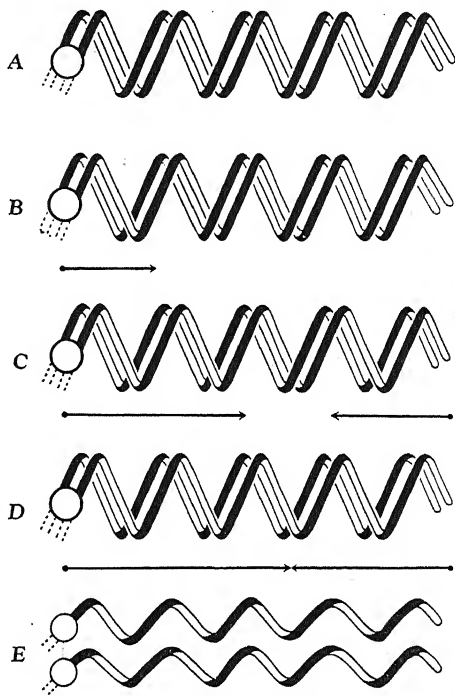


Fig. 25. A schematic illustration of the orientation of parallelisation of the spiral system. The circles represent kinetochores, and the arrows the direction of parallelisation. D and E are different aspects of the same spirals viewed from different angles.

## Discussion

### An hypothesis of the mechanism of crossing-over

That the paired chromatids of a metaphasic chromosome constitute the relational spiral system was first described by Kuwada ('38) in *Tradescantia*.<sup>1)</sup> In this paper he says (p. 19): "In *Trillium* such a case would not be expected to occur. If in *Trillium* in which the chromosomes in the second division are of the double coiled structure as in the first division and 'the first anaphase chromosomes pass without material change of structure to the second metaphase' (Huskins and Smith, 1935) such relational coils are formed between the sister chromatids,<sup>2)</sup> the chromosomes can not separate in the second division. In *Tradescantia*, on the other hand, this twisting around of the sister chromatids would afford no obstacle to separation in the second division, because in this plant the interkinesis exists in which the chromosome spirals are uncoiled or made loosely coiled, and in the second division the major spirals have been uncoiled." This statement is however at variance with the observed fact that in *Trillium* too its early metaphasic chromosomes show the same relational spiral relation of the paired chromatids. Furthermore Dr. Toyohuku of the writer's Laboratory has shown from careful observations on meiotic chromosome structure in *Tradescantia* that, although the paired chromatids of a metaphasic chromosome are relationally coiled except at the region of reversal in coiling direction they are already at early anaphase usually freely separated from one another except at the kinetochore region, thus giving half-bivalents of a cruciferous form, a condition entirely parallel to the case of *Trillium*. This finding leads to the implication that in *Tradescantia* the parallelisation process is retarded somewhat as compared with *Trillium*, that is, to early anaphase. He noticed also in *Tradescantia* the occurrence, though rarely, of chromosomes in the second metaphase in which the double-coiled structure still remains. It may accordingly be concluded that there is no essential difference in the parallelisation of spirals between *Trillium* and *Tradescantia*, except the time of its operation.

The mechanism of this parallelisation constitutes an important subject of the present discussion.<sup>3)</sup> The conversion of the relational

1) Darlington ('37) gives a figure clearly indicating the relational spiral in a bivalent of *Tradescantia* (Fig. 137 D), without making any reference to this point.

2) Kuwada employs the term "sister chromatids" in the place of "paired chromatids" in the present usage.

3) The bearing of the fact that the paired chromatids are relationally coiled at early metaphase on the mechanism of spiralisisation must be another important subject of discussion. This will be presented later elsewhere.



spiral system into the parallel one must take place under one of two conditions. Either it takes place by rotating the distal end of a chromosome in the direction opposite to that of the spiral, as many times as the number of twists, or else by the occurrence of segmental exchanges at twisting points without rotating the end. The first possibility can be excluded by the following considerations. First, the cause for such a rotation cannot be imagined, since it is considered as nothing but a teleological movement with the aim of parallelisation itself. Secondly, rotation is inconsistent with the fact that the parallelisation initiates also from the proximal end which is a fixed point and hence at which rotation is impossible.

It must therefore be concluded that the parallelisation process is associated with segmental exchanges between the two chromatids. How this is carried out is schematically illustrated in Fig. 26. Here the writer assumes that the major and the minor spirals respectively have their own matrices, adopting the view of Kuwada and Nakamura ('34). These may be termed respectively major and minor matrices.

The minor matrix is supposed to undergo division prior to the division of the major matrix. This division is most probably associated with the initiation of development of repulsions between the paired kinetochores and between the distal end regions of the chromatids, and with their advance towards the middle of the arm.

The division of the minor matrix (in the direction indicated by the arrow in Fig. 26A) will alter the three-dimensionally parallel condition of the two threads, a-a and b-b, into the two-dimensionally twisted condition at a certain point (Fig. 26B). At this cross-point the torsion stress due to the progress of matrix division will cause simultaneous breaks of the two chromatids which will be followed by reunions (Fig. 26C); such reunion following breakage is a common phenomenon in X-ray experiments. The reunion will so take place as to give rise to new chromatids, only in a longitudinal way (a1'-b2' and b1'-a2'), because the transversal join (a1'-b1' and b2'-a2') is rendered impossible by the cleavage of the matrix. The two chromatids, before the breakage, are intimately associated together by the minor coils which constitute a parallel spiral system from the beginning of their development.

This intimate association of the two strands will cause the break *accurately at corresponding points* in each of them. The resulting new chromatids, a-b and b-a, are then in a parallel spiral system (Fig. 26D), and this alternation of the system will cause the two strands to fall apart as far as possible within the major matrix. Thus the resulting contour of the spirals may be altered in their dimension

along the direction of the repulsion, as represented in Figs. 25D and E. The exertion of the repulsion between the two chromatids along their entire lengths will next cause the division of the major matrix,

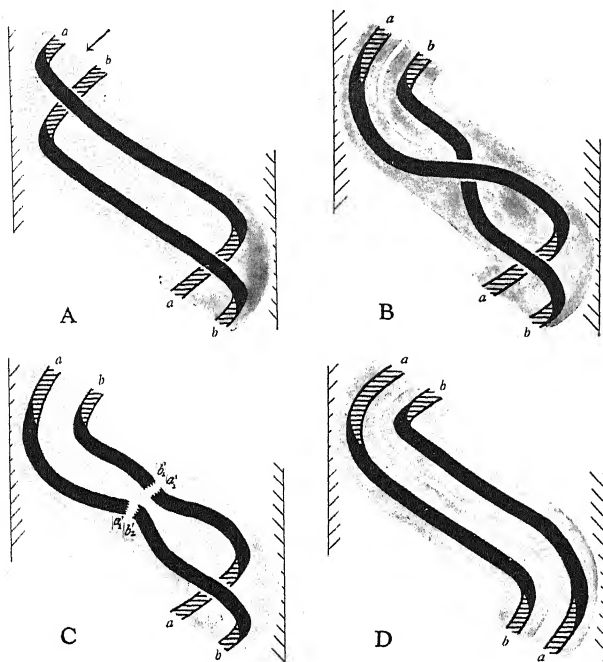


Fig. 26. Illustrating the mode of break and reunion between the paired chromatids. In this diagram only a half-coil is represented. The two chromatids are enveloped by the minor matrix (pale black) which is further covered by the major matrix (hatched). In order to simplify the diagram, the minor spiral is omitted.

Crossing-over is thus necessary consequence following the conversion of the three-dimensionally parallel (but two-dimensionally twisted) spiral system of the two strands into the two-dimensionally parallel (but three-dimensionally twisted) system.

In somatic mitosis each of the two chromatids of a chromosome usually takes an independent spiral system from the beginning of its development. Accordingly there will usually be no chance in mitosis of exchanges between them.

This interpretation of the crossing-over mechanism makes a marked contrast with all other earlier hypotheses which have considered it at early prophase of meiosis. It must be noticed however that although it was genetically proved that crossing-over takes place somewhere during the first reduction division, there is no definite

and in this way the half-bivalents of early anaphase come to take a cruciferous form, the chromatids falling apart and associating only at the kinetochore.

According to the writer's Neo-two-plane theory of bivalent constitution (see No. 4 of this series), the paired chromatids of each arm are non-sisters in two-thirds of the cases. Therefore in such cases, the segmental exchange between them results in detectable crossing-over.

proof that it should be at early prophase. If the present interpretation be adopted, the time of crossing-over is early metaphase in *Trillium* and early anaphase in *Tradescantia*. Probably certain other shifting of the time (e.g. to diakinesis, as supposed to be the case in *Zea* cf. Creighton and McClintock '32) may be shown in different organisms or under different environmental conditions even within the same species, according to different behaviors of the kinetochore in relation to the spiralisation of chromonemata.

The frequency and distribution of segmental exchanges between the two chromatids is the next problem to be dealt with in connection with their genetical consequences. Here there are many items of subjects to be discussed. Although full consideration on a purely theoretical basis will be presented elsewhere, some brief comments may be given here to show how justifiable explanations of the essential phenomena of crossing-over may be put forward in terms of the present theory.

1) The present theory explains as a matter of course a characteristic of crossing-over, that it takes place in the diploid at the four-strand stage of meiosis between only two of the four participating chromatids at any one point.

2) The present interpretation of the mechanism of break and reunion stands for the accurate correspondence in the exchange points between the two strands, which has been always genetically proved, with one exception in *Drosophila*.

3) In short chromosome arms less than the length of a half-coil, the chromatids can separate freely without any breakage. Probably the fourth chromosome of *Drosophila melanogaster* in which no cross-over is usually detectable will be an example of this case.

4) Generally speaking the break occurs once in each half-coil; therefore the frequency of exchanges is proportional to the number of spiral gyres (not strictly, *vide* item 5). Furthermore the number of spiral gyres per chromosome arm is exactly proportional to arm length (No. 1 of this series). Genetically these facts may imply that the relative distance between the genes is a function of the cross-over values and there is a certain correspondence of genetical map length with arm length (*vide* also item 6).

5) In the regions of the proximal and distal ends of an arm, one coil length of the chromatids may sometimes be freely separated without any breakage. The number of exchanges per chromosome arm must be between  $n$  and  $n-2$ , where  $n$  denotes the number of half-coils per chromosome arm. (The reduction of breaks due to the occurrence of reversals in coiling direction is here left out of

consideration.) This frequent failure of exchanges in both the end regions is parallel to the fact known in *Drosophila* that there is a greater amount of crossing-over per unit of material length in the middle of the X chromosome and also in the middle of the autosomes as compared with crossing-over frequency in the end regions.

6) Where  $n$  is small, the effect of the failure of exchanges in the end regions will be relatively greater than in cases where  $n$  is larger. Genetically this means that where the size difference is greater, the lack of proportionality between the genetical length and the actual chromosome length is more serious, a condition demonstrated in *Drosophila*.

7) The chromonema coiling is independent in the two arms of a chromosome (see Nos. 2 and 4 of this series). This may be taken as parallel with the fact that there is no interference between cross-overs in different sides of the kinetochore, as shown in the V-shaped autosomes of *Drosophila*.

8) Within a half-coil distance, two breaks will generally occur only rarely but beyond this limit one break will strongly tend to follow another. Genetically this means that a half-coil distance is the average interference distance and explains why interference is strong at adjacent regions but it vanishes and becomes even negative suddenly at a certain distance from the original point of crossing-over, as Kikkawa ('35) demonstrated in *Drosophila virilis*.

9) The process of parallelisation of the spirals which initiates at both ends of an arm and proceeds towards the middle will tend to shift the existing twists of the chromatids towards the central region. Thus the distance between two twists will be shorter in the central region than near the ends (see Figs. 5, 10, 21, and 22). Genetically it was demonstrated in *Drosophila* that the coincidence greatly increases near the middle region of the chromosome arm.

10) The priority of behavior in both the proximal and distal ends is entirely parallel to the conclusion drawn from *Drosophila* experiments by Charles ('38) that "crossing-over may be controlled jointly from the attachment and from the free end."

11) From the writer's Neo-two-plane theory it follows that the paired chromatids are non-sisters in 66% of cases. Therefore the highest recombination value to be expected will be 66%, though such a constant exchange at a definite region will be very rare. This seems to stand for rare cases of the occurrence of crossing-over more than 50%, e.g. about 60% cross-overs between G and S in *Pisum* (Wellensiek '29), which the other current theories of crossing-over fail to explain.

12) Since the length of a half-coil is the same in both the arms

of a chromosome and the force causing chromatid which is initiated first at the kinetochore works equally at both sides of the kinetochore, the frequency of breakage will tend to be high in regions symmetrically placed across the kinetochore. This seems to explain the case found in *Neurospora* by Lindegrens ('37).

13) The form of the spiral is greatly affected by environmental conditions such as temperature (see Nos. 1, 5 and 6 of this series). A fundamental difference in chromosome structure was shown in the chromosome type referred to as 'precocious' in the previous papers, in which the paired chromatids are coiled independently from the beginning of spiralisation as in mitotic chromosomes. Since there is no stage of the relational spiral in these chromosomes, there is no chance of material exchanges. Probably such is the case of *Drosophila* male or *Bombyx* female in which crossing-over is usually completely suppressed.

14) The mode of spiralisation under normal conditions is different in different organisms, even in allied species. The writer has shown that the meiotic chromosomes of *Paris quadrifolia* have more slender minor coils and more compact major coils than *Trillium kamtschaticum* (No. 11 of this series). Thus the absolute length of a half-coil is considerably less in *Paris*. Such structural difference may be related to the difference in the percentage of crossing-over in allied species, such as between *Drosophila melanogaster* and *D. virilis* (cf. Chino and Kikkawa '33).

15) If similar variation in chromosome structure is caused to occur in one organism by external agencies or in different sex, this will explain certain variations in crossing-over percentage shown under different environment conditions or by sex, e.g. *Primula*, *Zea*, *Apottetix*.

16) Under certain conditions the number of twists per chromosome arm is nearly constant (see item 5). It follows then that if some external factors happen to cause more frequent exchanges in a certain part of the chromosome, this will be naturally attended by a reduction of exchanges in the other part. This offers an explanation for the fact known in *Drosophila* and *Primula* that changes in crossing-over percentage are differential with regard to different parts of a chromosome, that is to say, weakening or strengthening of the linkage between two genes of a group is accompanied by correspondent strengthening or weakening of the linkage between other genes of the group.

17) Such changes in exchange frequency may be attributed to certain timing unbalance in the parallelisation process, that is, between the behavior of the kinetochore and that of the distal end.

Abnormal external conditions may cause retardation in the separation of the associated kinetochores. If their separation is so retarded that the parallelisation is completed only in the direction from the distal end, the exchanges will naturally be increased in the neighbourhood of the kinetochore. Really it was found that crossing-over is increased in the kinetochore region of the X chromosome and of the autosomes of *Drosophila* by changes in temperature and by X-rays.

The present study was aided by a grant from the Hattori Hôkôkwai, to which the writer wishes to express here his sincere thanks.

### Summary

1) The paired chromatids of each meiotic chromosome constitute at early metaphase the relational spiral system (Fig. 1A), while those at late metaphase take the parallel system (Fig. 1B).

2) This conversion of the spiral system takes place first from the proximal region and then independently from the distal end. These processes proceed inwards along the arm and when they meet one another the parallelisation is completed.

3) Such an orientation of the parallelisation excludes a possibility that the system is altered by rotation of the free end. The other possibility must, therefore, be admitted that the parallelisation is associated with breaks and reunions between the chromatids at twisting points existing in the spiral system. This breakage is supposed to be caused by the cleavage of the matrix enveloping the minor spiral, which at the same time plays a rôle for preventing the transversal reunions of the broken ends (Fig. 26).

4) According to the writer's Neo-two-plane theory, the paired chromatids are non-sisters in two-thirds of the cases. Therefore in these cases such breaks and reunions should result in detectable cross-overs.

5) This new hypothesis of crossing-over mechanism presents reasonable explanations for many essential characteristics hitherto known in the crossing-over phenomena.

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## Effects of Fast Neutrons upon Plants, II. Abnormal behavior of mitosis in *Vicia faba*

By

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(With 46 figures in the text)

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A number of experiments with neutron rays have been carried out on both animals and plants and certain biological effects have already been reported. We ourselves have established in the case of two plant species, *Fagopyrum esculentum* and *Cannabis sativa* that the neutron irradiation produces abnormalities not only in external morphological features but also in chromosomal behaviors (cf. Nishina and Sinotô 1939; Nishina, Sinotô and Satô 1940). But the effect of neutron rays on the morphology and the behavior of chromosomes in the mitotic or meiotic cycle has not so far been analysed in detail, and therefore the present work was undertaken. *Vicia faba* was used as material, because this plant is familiar in the cytological laboratory and has larger chromosomes which facilitate easier and more effective observation. In the present work three different effects of irradiation on mitosis, namely, primary effect, mitosis-free period effect and secondary effect (cf. Politzer, 1934; Marquardt 1937, '38) have been analysed and the number of mitotic figures in different stages has been dealt with statistically.

### Material and methods

Seeds of *Vicia faba* after having been immersed in tap water for about 24 hours were planted in saw-dust in pots. The germinating seeds were then placed in a metal box, which was inserted in the irradiation chamber of the cyclotron in this Laboratory, and were exposed for 20, 30 and 60 minutes to neutron rays obtained by bombarding a beryllium target with 2.8 MeV deuterons from the cyclotron. The root-tips were then fixed at 0, 12, 23, 40, 96 and 120 hours respectively after the termination of the exposures. The seeds were classified into three groups, III (strong), II (medium) and I (weak), according to their distance from the target, namely 1-4, 4-8 and 8-12 cm respectively. The intensity of the neutron rays was estimated by reference to the radioactivity induced in a thin sheet of red phosphorus placed in front of the samples and was expressed in an arbitrary unit.

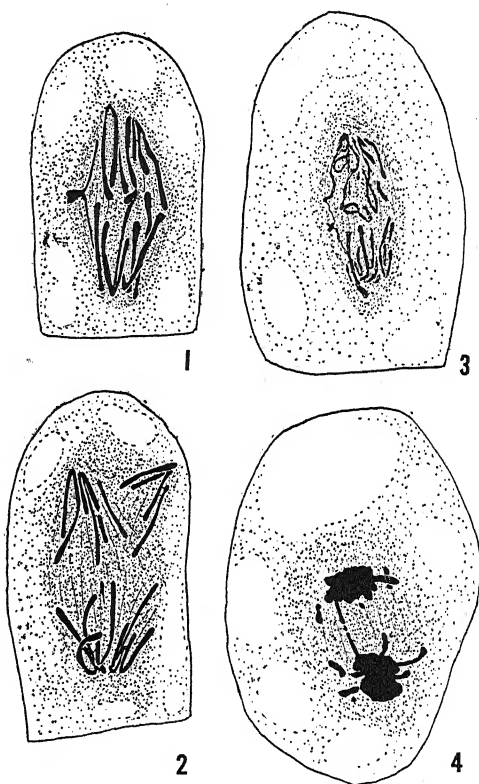


The root-tips were fixed with the Bonn modification of Flemming's solution. The paraffin sections were cut 15–20  $\mu$  in thickness and subjected to the double staining method with gentian violet and light green.

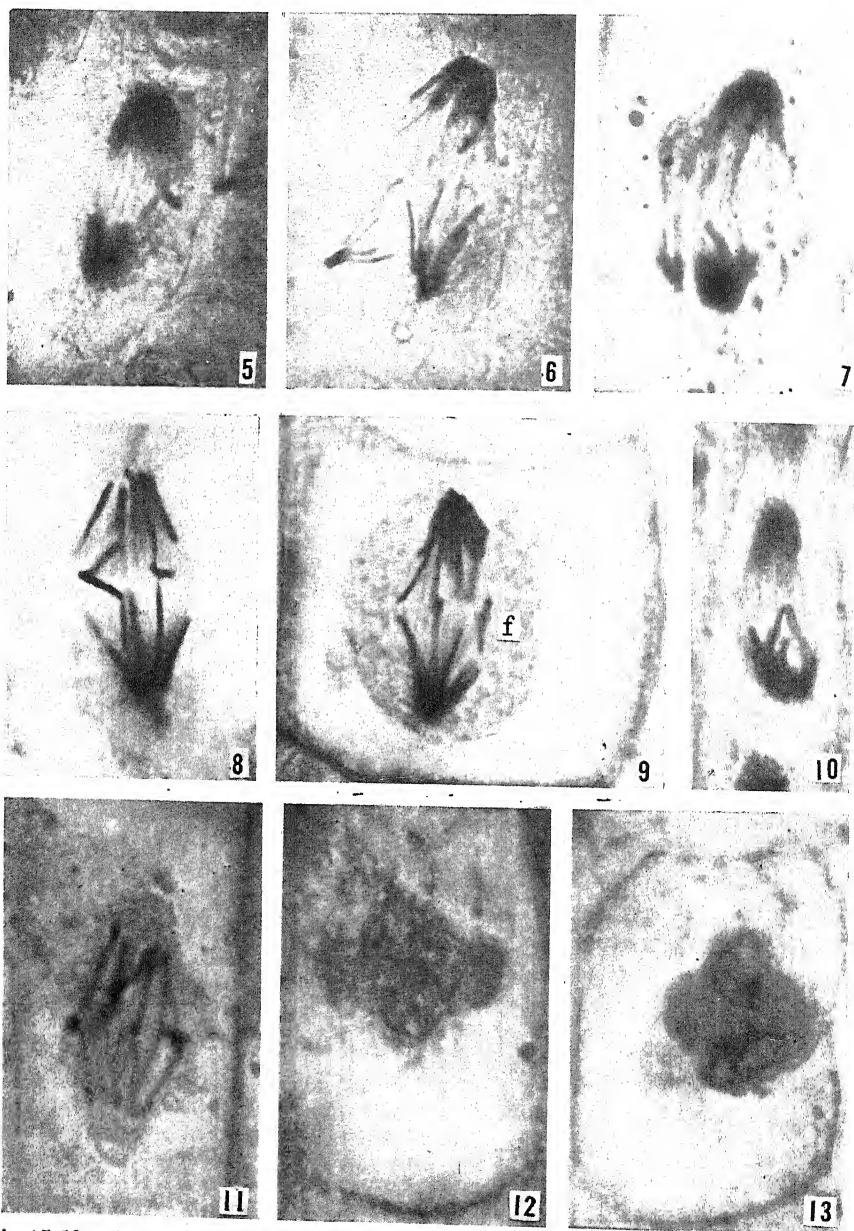
### Cytological observations

#### (A) Fixed immediately after irradiation (figs. 1–13).

When root-tips were fixed immediately after neutron bombardment for 20 minutes, mitotic divisions in the root-tip cells hardly showed any visible abnormalities in the case of the weak treatment (I), but more critical observations showed some irregularities such as breaks or fusions of the distal arms of separating chromosomes in the anaphase and an enormous increase of the telophase configurations (tab. 1). It is evident accordingly that the weak treatment can induce a few chromosome aberrations and advance the mitotic process from metaphase to telophase, the latter being consequently most frequent. This advancement of the mitotic process seems to bear an intimate relation to the cytoplasmic condition such as "Entmischung" (cf. Bělař 1930, Wada 1936, '39) or vacuole formation which may cause deformation of the mitotic spindle (cf. Wada, 1936, who observed such a rapid elapse of the mitotic process from the anaphase to the telophase in *Tradescantia* stamen hairs.) When a vacuole is formed near the spindle



Figs. 1–4. Mitotic figures of the root-tip cells in *Vicia faba* showing breaks and fusions of chromosomes immediately after irradiation for 30 minutes (300 units). 1, 2, weak treatment (I) and 3, 4, strong treatment (III). 1, subterminal fusion of distal arms of separating chromosomes. 2, tripolar division probably resulting from vacuole formation near the atractosome. Drawn from fig. 6. 3, 4, fusions and breaks of distal arms of separating chromosomes. Vacuole formation and weak stainability are clearly seen in strong treatment (III).  $\times 1260$ .



Figs. 5-13. Anaphases and telophases of root-tip cells fixed immediately after irradiation showing breaks and fusions of chromosomes and tripolar division (division of polar region). 5, 6, irradiation for 30 minutes, weak treatment (300 units, I), 7-11, for 20 minutes, medium treatment (II) and 12-13, strong treatment (III). 5, fragments remaining at the equator in the early telophase. 6, 7, division of polar region. Fig. 6 is the same as fig. 2. 8, subterminal fusions of distal arms of two separating chromosomes are clearly shown. These figures show a chromatid break at the corner of chromosome or at the point of fusion. 9, subterminal fusion of distal arms of separating chromosomes at the centre and two fragments (f) at the right

(atractosome), a tripolar division may secondarily occur (fig. 2, 6, 7). Such divisions and free fragments were rarely observed, while subterminal and terminal fusions of the distal arms of separating chromosomes were of common occurrence among the induced abnormalities. Abnormalities were more marked in the case of neutron irradiation for 30 minutes (300 units) than in the case of treatment for 20 minutes.

In the case of medium treatment (II) abnormalities were more conspicuous than in the case of weak treatment (I) and single breaks or fusion of non-homologous chromosomes were occasionally observed (fig. 10), while fusion of the distal arms of separating chromosomes was commonly observed. In the extreme cases almost all of the chromosomes fused at the distal arms in the anaphase (fig. 11) and many non-homologous chromosomes also fused with each other to form a chromosome complex. Moreover, the effects of neutron bombardment result in an enormous increase of mitotic figures in certain stages. On one hand it advances or stimulates the chromosome formation from the spiremes in the prophase and increases metaphase configurations. On the other hand many abnormalities of the chromosomes may disturb the rapid elapse of the mitotic process during anaphase and telophase and result therefore in an increase of the mitotic figures in these two phases. The effect on the cytosome is more marked than in the case of weak treatment I and a vacuole formation and "Entmischung" are frequently observed, while a decrease in the stainability of the chromosomes was clearly observed, especially in the anaphase.

In the case of strong treatment (III) the mitotic figures showed more irregularities and the stainability of the chromosomes was so markedly reduced that the mitotic figures in the anaphase could hardly be observed (fig. 3). In the prophase, clumping of the chromosomes could be observed, and in the extreme case all the chromosomes were fused with each other at the equator inside the atractosome; this nucleus indeed might have resulted in a syndiploid one.

The following abnormalities were observed in the late telophase. In one case, two daughter nuclei situated close to each other clearly show a phragmoplast developing between them (figs. 12, 24). In another case, two chromosome bridges are found between two near daughter nuclei (fig. 13). The latter suggests that the mitotic pro-

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are clearly shown. 10, terminal fusion of distal arms of non-homologous chromosomes in the telophase. 11, subterminal fusion of distal arms of several chromosomes. 12, two daughter nuclei remain closely, the phragmoplast forming (cf. fig. 24). 13, two daughter nuclei still closely situated, showing two chromosome bridges between them.  $\times 1400$ .

cess from anaphase to telophase can take place in the brief period of 20 minutes in the case of the strong neutron treatment. These figures in the late telophase are correlated with the formation of large vacuoles in the cytosome and the fusion of daughter nuclei is the secondary product of this cytoplasmic effect of the neutron bombardment. In the case of strong treatment for 30 minutes (300 units, III), both resting nucleus and cytosome underwent damage, and clumps of the nucleus or of both nucleus and cytosome showed necrobiotic figures.

Table 1. Total numbers of mitotic figures in different stages in six root-tips of *Vicia faba* exposed to neutron rays for 20 minutes and immediately fixed. Numbers of abnormal mitotic figures are put in brackets. Weak, medium and strong treatments of each exposure to neutrons are indicated as I, II, and III respectively.

	Total	Metaphase	Anaphase	Telophase
Control	1389 (0)	594 (0)	430 (0)	365 (0)
I <sub>1</sub>	1345 (42)	339 (0)	295 (41)	711 (1)
I <sub>2</sub>	1192 (59)	380 (8)	230 (35)	582 (16)
II <sub>1</sub>	1565 (232)	655 (13)	361 (205)	535 (14)
II <sub>2</sub>	3001 (708)	954 (7)	992 (667)	1055 (34)
III	1822 (521)	571 (6)	568 (499)	683 (16)

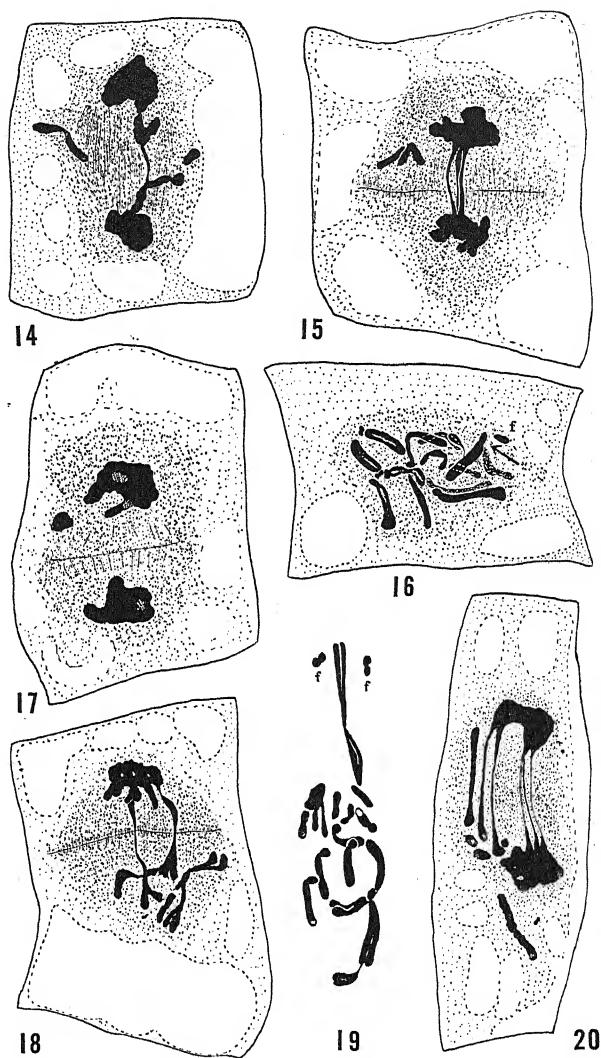
Table 2. Total abnormal chromosome numbers of the anaphase of root-tips of *Vicia faba* exposed to neutron rays for 20 minutes and then immediately fixed.

	Total cells	Total chromosomes	Single break	Division of pole	Fusion	Complex
I	295	3540	1	0	81	0
II <sub>1</sub>	361	4332	63	3	315	6
II <sub>2</sub>	992	11904	106	4	1370	43
III	568	6816	30	0	1188	4

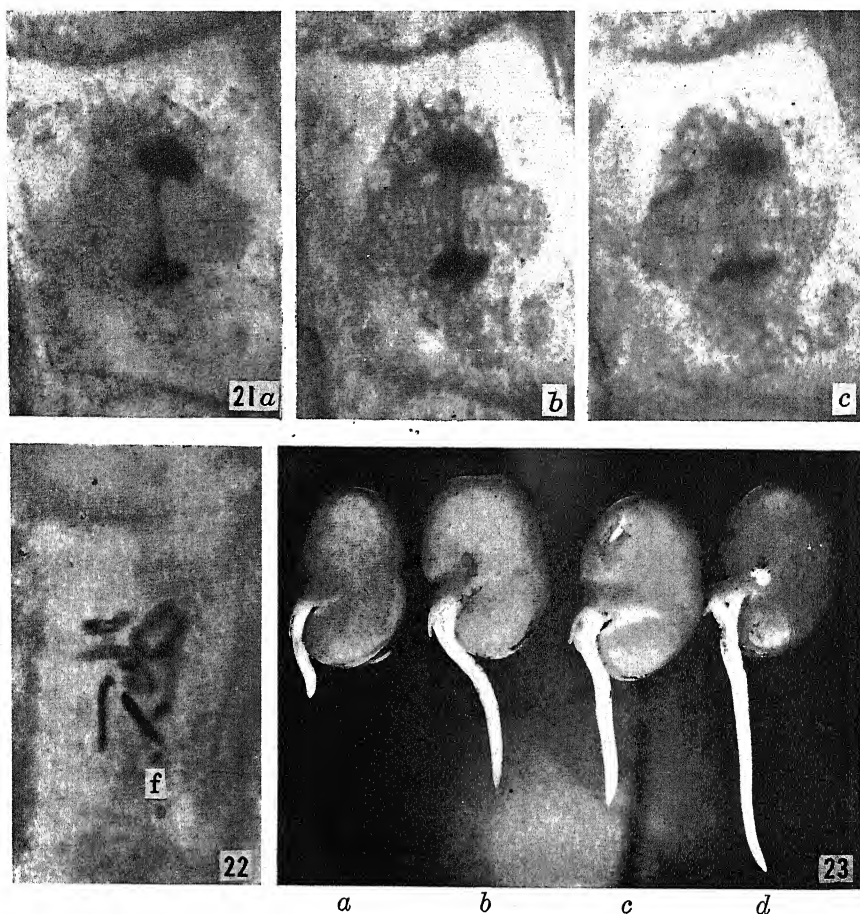
(B) Fixed 12 or 23 hours after irradiation (figs. 14-23).

Root-tips exposed for 20 minutes were fixed at the end of 12 hours after treatment and those exposed for 30 minutes (300 units) were fixed at the end of 23 hours after treatment. The mitotic figures in the root-tips decreased markedly in the exposed material compared with the findings in the control one. The disturbance of the growth of the root-tips is correlated with such decrease of the mitotic figures in the exposed material (fig. 23). These mitotic figures showed many abnormalities in the anaphase and telophase or more properly speaking, only abnormal mitotic figures remained in these stages while the normal ones seemed to have progressed more rapidly to the resting stage. The most common abnormality in the anaphase was the chromosome bridge. Free fragments, stray chromosomes (figs. 14, 15) and micro-nuclei were frequently observed in the telophase. Many

chromosome bridges with or without free fragments could be observed. Such bridges can break at any point, but their middle section near the phragmoplast is the one most frequently broken. These abnormalities may have their origin in the fusion of sister spindres or chromatids in the prophase or the resting stage. When many chromosome bridges exist in one and the same cell, two daughter nuclei may form a fused nucleus. A chromosome complex is also observed and a fragment is apt to become attached to other chromosomes (figs. 16, 22), especially to the chromosome ends or points of breaks. Chromosome fragments were always observed, but chromatid fragments (shown by arrow in figs. 16, 22) could not be so easily detected, which seems to show that the effect of the



Figs. 14-20. Mitotic figures observed 23 hours after irradiation for 30 minutes (300 units). 14, 15, 17, weak treatment (I), 16, medium treatment (II) and 18-20, strong treatment (III). 14, 15, chromosome bridges and stray chromosomes or fragments in the telophase (cf. fig. 21 from which fig. 15 is drawn). 16, lateral fusion of a fragment to the deficient chromosome showing chromatid fragmentation, drawn from fig. 22 (Note especially an arrow). 17, abnormal telophase having micro-nucleus. 18, 20, chromatid bridges, fused chromosomes and fragments are shown in the telophase. A mitotic figure such as shown in fig. 20 may form a fused nucleus or a syndiploid one. 19, abnormal metaphase plate showing two fragments and one chromosome complex (fusion of several chromosomes).  $\times 1260$ .



Figs. 21-23. Mitotic figures and root-tips of *Vicia faba*, 23 hours after irradiation for 30 minutes (300 units). 21, *a-c*, photographs taken in three different foci from an anaphase figure showing three chromosome bridges and two stray chromosomes (cf. fig. 15). 22, lateral fusion of a fragment to the deficient chromosome releasing a chromatid fragment (cf. fig. 16, especially note an arrow). 23, Four seedlings showing different growth of their root-tips. *a*, strong (II), *b*, medium (II) and *c*, weak (I) treatments and *d*, control. 21-22.  $\times 1400$ , 23.  $\times 1\frac{1}{2}$ .

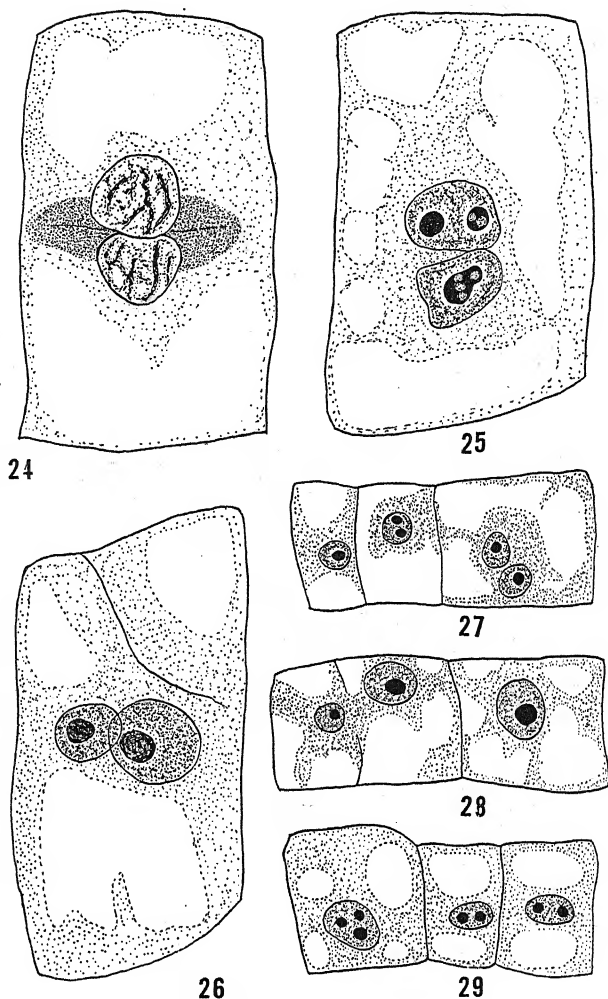
stronger bombardment of neutron rays was in our case to induce chromosome fragments rather than chromatid ones.

Table 3. Numbers of total and abnormal (indicated in brackets) mitotic figures in different stages of root-tips of *Vicia faba* exposed to neutron rays for 30 minutes (300 units) and fixed 23 hours after treatment. Weak, medium and strong treatments are indicated as I, II and III respectively.

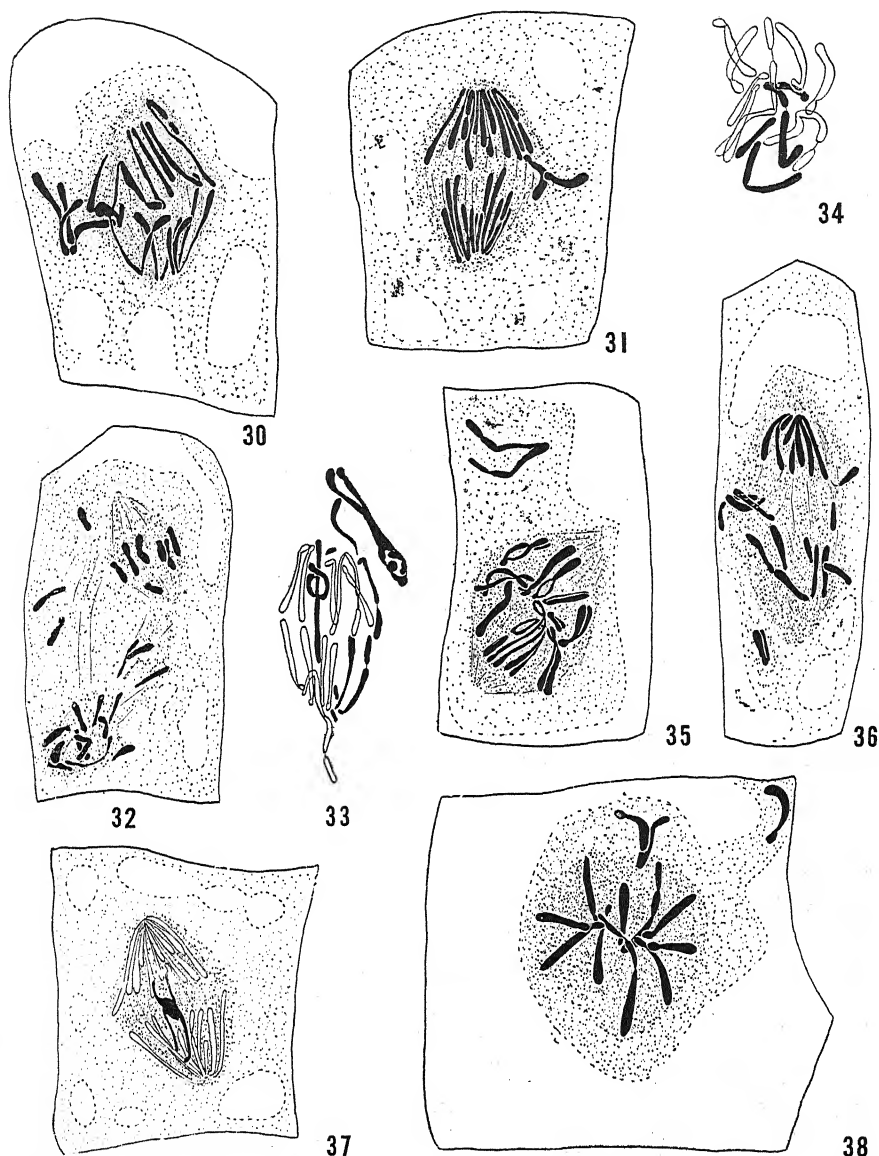
	Metaphase	Anaphase	Telophase	Resting stage
Control	594 (0)	430 (0)	365 (0)	—(0)
I	133 (13)	61 (40)	155 (104)	—(4)
II	12 (3)	5 (4)	27 (27)	—(2)
III	2 (1)	0 (0)	10 (10)	—(0)

(C) Fixed 40 hours after irradiation (figs. 24-29).

The number of mitotic figures was reduced at the end of 40 hours after irradiation for 30 minutes (300 units) and abnormalities were only found in the resting stage. Large cells with either two nuclei or only one large nucleus were found among small normal cells. An irregular cell plate formation was found in a cell with both large and small nuclei. This abnormality may be derived from the abnormal mitosis, followed by abnormal behaviour of the phragmoplast due to vacuole formation. In fig. 24 two daughter nuclei still remain close to each other, probably on account of vacuole formation in the cytosome; thus the rudimentary phragmoplast or cell plate may disappear when it is prevented from arriving at the cell



Figs. 24-29. Abnormalities found in the late telophase and the resting stages after irradiation. 24, approach or fusion of two daughter nuclei probably owing to the preceding vacuole formation clearly showing the existence of phragmoplast (fixed immediately after treatment (III) for 20 minutes, cf. fig. 12). 25, two near daughter nuclei, showing absence of the phragmoplast. 26, abnormal cell with large and small nuclei showing an irregular membrane formation. 27, a large cell with two nuclei of a similar size and two small normal cells. 28, an abnormal cell with large and small nuclei having an irregular membrane, and another cell with a large fused nucleus. 29, an abnormal cell with a large fused nucleus having three nucleoli, and two small cells with normal nuclei having two nucleoli respectively. 25-29, fixed 40 hours after irradiation for 30 minutes (300 units). 24-26.  $\times 1260$ , 27-29.  $\times 420$ .



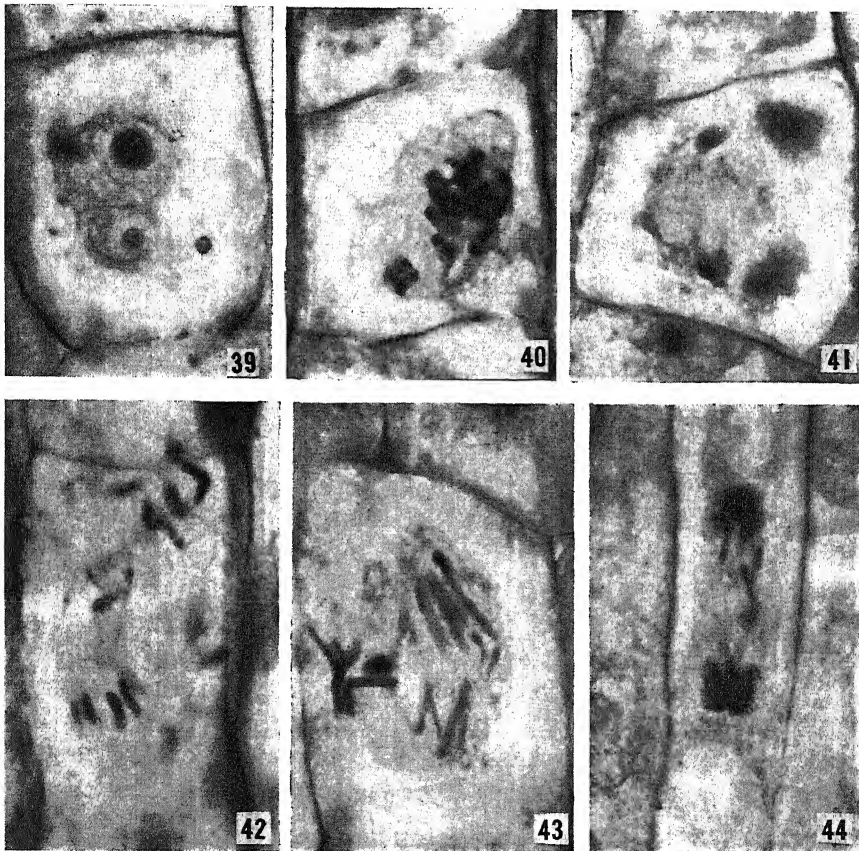
Figs. 30-38. Abnormal mitoses seen 96 hours after irradiation for 60 minutes (340 units, weak treatment). 30, one chromosome complex probably made of three chromosomes and abnormal chromosomes without visible longitudinal splitting, thrown out into the cytosome from the atractosome (drawn from fig. 43). 31, two abnormal chromosomes without longitudinal splitting being thrown out into the cytosome. 32, irregular spindle formation from a poly-nucleate cell (drawn from fig. 42). 33, two chromosome complexes and fragmented V-shaped chromosomes are clearly seen. 34, two short chromosomes with short deficiencies, fragments of which translocated to other two long chromosomes. 35, eleven chromosomes showing a normal metaphase plate, while one V-shaped chromosome showing a terminal fusion of chromatids thrown out from the spindle. 36, irregular mitosis. 37, anaphase showing a chromosome complex. 38, one chromosome being at a distance may have come from a micronucleus and two other chromosomes may have been thrown out into the cytosome from the atractosome.  $\times 1260$ .



wall by the abnormal vacuole formation (fig. 25). The phragmoplast is driven away from the ordinary position and forms an irregular incomplete cell plate (fig. 26). With these abnormal features known, figs. 27-29 are almost self-explanatory. The large cells in figs. 28 and 29 show syndiploid nuclei which may have arisen either from irregular mitosis with many chromosome bridges or from the fused nucleus in the preceding metaphase or anaphase.

(D) Fixed 96 or 120 hours after irradiation (figs. 30-44).

The mitotic figures again increased in the material from the treatment I, fixed at the end of the 96 hours after irradiation for 60 minutes (340 units), when compared with the case



Figs. 39-44. Abnormalities appeared 96 hours after irradiation for 60 minutes (340 unites, weak treatment). 39, approximation or fusion of large and small nuclei and one micro-nucleus in the resting stage. 40, large and small nuclei in the prophase. 41, mitotic figures in poly-nucleate cell. 42, abnormal mitosis in poly-nucleate cell (cf. fig. 32). 43, a chromosome complex and abnormal chromosomes thrown into the cytosome out of the atractosome (cf. fig. 30). 44, lagging chromosomes in the telophase.  $\times 1400$ .

of waiting 12, 23 and 40 hours. This might be attributed to recovery from the effect of neutron bombardment or to the changed periodicity of mitosis induced by irradiation. Abnormalities such as stray chromosomes, chromosome fusion, fragmentation, translocation and chromosome complex were observed in the metaphase and anaphase. Judging from the abnormalities thus observed the effect of neutron bombardment seems to have been exercised on the preceding division or the resting stage. When the extra micro-nucleus which was made abnormally in the preceding mitosis is present, either two mitotic figures are formed in a cell (figs. 35, 38, 41) or the mitosis is entirely irregular (figs. 32, 42).

The stray chromosomes, showing no visible longitudinal splitting, seem to arise not on account of kinetochore deficiency but rather on account of fusion of the kinetochores (figs. 30, 31, 43). The fusion of the kinetochores might be induced directly or indirectly by the impact of ionizing particles in the preceding division or resting stage.

### Discussion

After neutron bombardment on normal mitotic tissues, as in some of the cases of X-raying (cf. Politzer 1934; Marquardt 1937, 1938), three different effects are recognized, namely, a primary effect (0-24 hours after irradiation), a mitosis-free period effect (about 24-72 hours or more after irradiation) and a secondary effect (over 96 hours after irradiation).

The primary effect appears after the treatment of the cells entering into mitosis and results in fusion and fragmentation of chromosomes and a decrease in the stainability of the chromosomes with gentian violet.

The statistical analysis of mitotic figures of the root-tips fixed immediately after neutron bombardment showed interesting results, namely, advancement of the mitotic process from metaphase to telophase, and at the same time advancement of the chromosome formation from prophase spiremes or chromonemata and delayed mitoses from metaphase to telophase owing to chromosome abnormalities (agglutination of chromosomes and delayed splitting of kinetochores, etc.).

Such statistical investigation of the effect observed in the material fixed immediately after irradiation does not yet seem to have been undertaken even in the case of X-raying, whereas the secondary effects were reported in *Secale* (Breslavetz and Afanassieva 1937), *Pisum* (Atabekova 1937) and *Triticum* (Afanassieva 1938). The last named author stated that the number of mitotic figures decreases

as the X-ray dosages increase, and attributed this to a reduction of the prophase nuclei number, because she found no reduction in numbers of mitotic figures in other stages. In the case of the present observation, however, the relative numbers of the mitotic figures in different stages were found very different from those of the non-irradiated sample and also vary considerably according to the doses (cf. Tables 1 and 2, figs. 45 and 46).

The advancement of the mitotic process from metaphase to telophase was found in the case of living *Tradescantia* stamen hair by Wada (1936) who treated the material by desiccation which necessarily induced dehydration of the chromosomes. In the case of ammonia-chloroform mixture, the effect on the mitotic figure at first brings hydration of the chromosomes and spindle substance and then the chromosomes change over to the resting nucleus structure and the spindle substance to the phragmo-plast-forming substance (cf. Wada 1939). The changes in colloidal states, leading to the morphological aberrations, of the chromosomes and surrounding substances seem to be a result of their ionization effected by neutron

irradiation. In view of the results of physico-chemical investigations (cf. Wada 1939, Kuwada 1939) the ionization of neutrons in the case of the metaphase and anaphase probably accelerates dehydration of the chromosomes and in the case of the prophase hydration (see below). The effect of ionization on the cytoplasm possibly

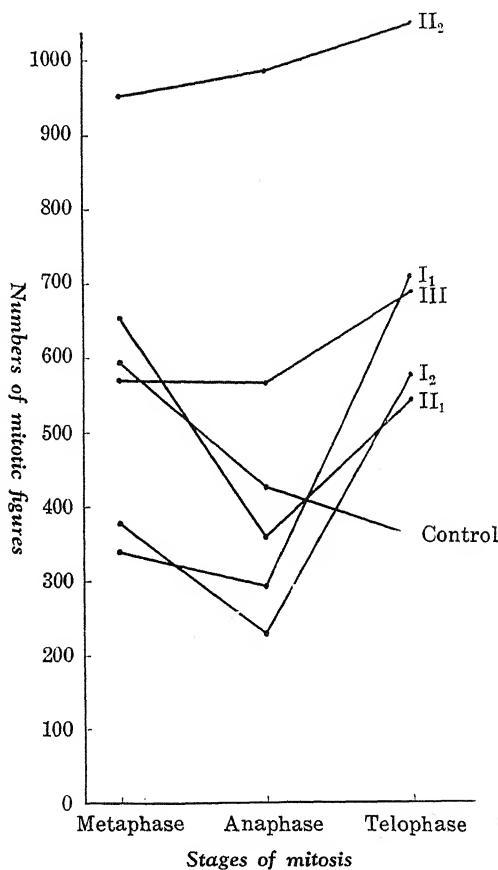


Fig. 45. The relation between numbers of mitotic figures and different stages of mitosis of the root-tips of *Vicia faba* irradiated for 20 minutes and immediately fixed. Weak, medium and strong treatments are indicated as I, II and III.

causes "Entmischung" and vacuole formation (and similar changes may also occur in the atractoplasm) and abnormal spindle figures (sometimes tripolar divisions).

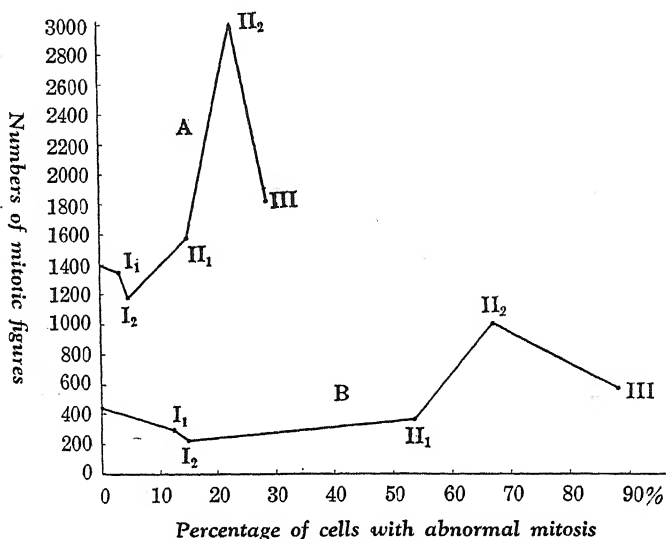


Fig. 46. The relation between numbers of mitotic figures and per cent cells with abnormal mitosis in the root-tips of *Vicia faba* irradiated for 20 minutes and immediately fixed. Weak, medium and strong treatments are indicated as I, II and III. Curve A showing total mitotic figures and curve B mitotic figures found only in the anaphase.

The delayed mitosis seems to be interrelated to some extent with the effect of the neutrons on the chromosomes, namely clumping of chromosomes and delayed splitting or fusion of kinetochores. The mitotic process is delayed by irradiation as abnormalities of the chromosomes increase or the treatment is intensified. In the case of the treatments II and III the accumulation of mitotic figures in the anaphase and telophase shows the retardation of the later stages of the mitotic process. The passage of ionizing particles through chromosomes probably induces or causes fusion, breakage, simple or reciprocal translocations and chromosome complexes. A decrease in the stainability of the chromosomes with gentian violet suggests a change in the chemical nature of the chromosomes. For the understanding of the chromosome aberrations, however, the change of the conditions caused by irradiation in the whole protoplast must also be taken into consideration. These chromosome alterations have also been induced by various other agents such as X-rays and low temperature, etc. The delayed separation of the kinetochore induced by colchicine or acenaphthene treatments is clearly different from the present case which does not show characteristic c-pairing.

The advancement of the chromosome formation from prophase spiremes or chromonemata is a characteristic feature of neutron irradiation. Such a process can only be observed otherwise in the case of treatment with some chemicals such as *n*-butyl alcohol, ammonia and colchicine, etc., while the prophase nuclei are apt to return immediately to the resting stage in the case of some other treatments such as chloroform, ether, etc. (cf. Wada 1939).

The nucleus in the prophase is sensitive to external agents and a return to the resting stage is commonly observed in the case of the usual chemical or physical stimuli, and only nuclei in the late prophase continue the mitotic process. This condition of restitution of the prophase nuclei seems to vary according to the nature and strength of the agents. In the case of colchicine treatment, for example, nuclei in the early prophase do not in general return to the resting stage and the mitotic process can continue under the influence of this agent in rather strong concentrations. As for neutron irradiation it activates the formation of chromosomes from prophase spiremes and results in an increase in the number of metaphase plates and a decrease in the number of early prophase nuclei, partly due to the probable returning to the resting stage.

The process of chromosome formation from prophase chromonemata is hydration of chromosomes, that is the reverse of the process which occurs during metamorphosis from the metaphase to the telophase, the direction of the process being determined by the conditions of the medium or the surrounding substances. These two processes thus occur even in neighbouring cells undergoing the same treatment, for we can easily understand the difference of the conditions in the prophase from those of the other stages (cf. Wada 1939).

The mitosis-free period increases in duration as the neutron- or X-ray dosage increases. Cells already in the prophase continue division, and a period of quiescence then follows. The mitosis-free period is very pronounced in vegetative tissues (growing roots, etc.), but is almost negligible in both meiosis and pollen grain mitosis (Marquardt 1938; Sax 1938). The occurrence of a mitosis-free period probably implies that chromosome reproduction is prevented or delayed on account of the changes of the condition by irradiation in the whole protoplast.

The secondary effects comes from those cells which went through the mitosis-free period, that is either from those affected and stayed in the resting stage or from those attacked during the preceding mitosis and proceeded to the next resting stage. This is seen in cells starting the prophase, only after exposure to weak neutron bombardment

(340 units, I). Fragmentation, fusion and translocation of the chromosome occur, resulting in the formation of free or attached fragments and chromosome bridges. As regards the fragments, three types may be differentiated, namely identical and different chromosome fragments (chromatid piece of equal or different size) and chromatid fragments. In the case of the present observation in the dividing roots, where there is a pronounced mitosis-free period, the resting nuclei produce chromosome fragments exclusively (cf. Gentscheff and Gustafsson 1939).

### Summary

Three different effects (the primary, mitosis-free period and the secondary effects) of neutron bombardment on the root-tips of *Vicia faba* were described cytologically. The primary effect consists of advancement of the mitotic process from metaphase to telophase, retardation of the same mitotic process owing to the chromosome aberration and relative advancement of chromosome formation from prophase chromonemata. The former may chiefly be attributed to the effect on the cytosome and chromosome (dehydration) which resulted in an increase in the number of mitotic figures in the telophase, while the latter two are attributed to the effect on the chromosomes, namely fragmentation, clumping and hydration. These, moreover, result in an accumulation of mitotic figures in the metaphase and later stages.

The mitosis-free period results from the delay in chromosome formation from the resting nuclei, induced after irradiation.

The secondary effect, which reveals itself after the mitosis-free period, shows abnormalities appearing in the process of recovery of cells from the effect of the neutron bombardment. Fragmentation and fusion of the chromosomes and irregular mitoses were observed 96 hours after irradiation.

The fragments formed were chiefly chromosome fragments and rarely chromatid fragments. The abnormal changes in somatic mitoses produced by the action of neutron rays may be comparable with those seen in the case of the desiccation treatment (cf. Wada 1936).

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Nuclear Research Laboratory,  
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Div. of Genetics, Bot. Inst.,  
Fac. of Sci., Tokyo Imp. Univ.

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## Verschiedenartige Entformungen entquellender Ciliaten

Von

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(Mit 12 Abbildungen im Text)

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Bei der Entquellung, die in der von mir angegebenen Methode (KLEIN, 1926, 1937/38) zur Darstellung des Silberlinien- oder neuroformativen Systems eine grundlegende Rolle spielt, läßt sich, was die Erhaltung der Form der ihr ausgesetzt gewesenen Tiere anlangt, die merkwürdige Feststellung machen, daß die betreffenden Tiere sich in dieser Beziehung recht verschiedenartig verhalten. Einmal, es ist die Mehrzahl der Fälle, zeigen sich besterhaltene Formen, die von den ursprünglich im Leben vorhanden gewesenen Verhältnissen nur dadurch abweichen, daß durch Wasserabgabe, die dritte Dimension des Tieres, gegenüber den beiden anderen, durch die feste Unterlage des Tragglases gestützten Dimensionen, praktisch verschwindet, in den letzteren sozusagen aufgeht, so daß die Tiere flach werden, auf eine Ebene reduziert erscheinen, die bei Untersuchung mit hohen Vergrößerungen den Wert einer optischen Ebene besitzt. Neben dieser guten, ungestörten Formerhaltung treten aber, wenn auch nur viel seltener, die verschiedenartigsten Entformungen auf und zwar so, daß solche, die offensichtlich in ganz verschiedener Weise entstanden sind, knapp nebeneinander und neben nicht entformten Tieren im gleichen Präparat liegen. Schon eine oberflächliche Betrachtung läßt die Vermutung aufkommen, daß die verschiedenen Entformungstypen nicht ohne weiteres oder überhaupt nicht auf gemeinsamen Nenner zu bringen sein werden, d.h., daß sie durch verschiedene Ursachen bewirkt werden bzw. jeweils verschiedenen Zuständen des lebenden Zellplasma's ihr Entstehen verdanken. Die vorkommenden Entformungen in diesem Sinne als Anzeiger verschiedener äusserer Ursachen bzw. individueller Zustands-Verschiedenheiten der einzelnen, gleichzeitig der Entquellung ausgesetzt gewesenen Tiere zu werten und sie nicht nur rein äußerlich zu beschreiben, versuchen die folgenden Aufzeichnungen. Daß bei der Vermittlung der diesbezüglichen Tatsachen Bilder, und zwar mikrophotographische Aufnahmen, die wichtigste Rolle spielen, ist wohl selbstverständlich, da nur sie einen geschlossenen, durch Worte allein nicht zu erreichenden Eindruck geben können.

Damit die Bilder jeweils eine Gesamt-Übersicht der Formver-

hältnisse geben können, mußten die ausnahmsweise oft sehr plastischen Objekte bei einer mittleren Einstellung aufgenommen werden, der natürlich die Schärfe mancher hier nebensächlicher Einzelheiten zum Opfer fiel. So mußte nicht Rücksicht genommen werden auf die unbedingt scharfe Abbildung des Silberliniensystems, besonders dort, wo die Entformung an ihm weder strukturelle noch formative Reaktionen zeitigte. Aber auch dort wo dies der Fall ist, wurde, da es unmöglich ist nach beiden Richtungen befriedigende Ergebnisse zu erzielen, vor allem auf die charakteristischen Entformungen Rücksicht genommen, umsomehr, als im allgemeinen die Art der hier am Silberliniensystem vorkommenden strukturellen und formativen Reaktionen grundsätzlich die gleiche ist, die in früheren Arbeiten (KLEIN, 1927) ausführlich beschrieben und durch scharfe Bilder belegt ist. Das Silberliniensystem ist jedoch in anderer Beziehung für die hier gegebenen Bilder bzw. die vorliegende Untersuchung überhaupt, nicht belanglos: Sollen nämlich Entformungen einer Zelle festgestellt werden, die nicht schon der Zellumriß verrät, was z.B. bei Verschiebungen oder Verdrehungen einzelner Zell- bzw. Ektoplasmapartien gegeneinander ohne Störung des Gesamtzellumrisses der Fall ist, so ist dies nur möglich, wenn jeder Ort der Oberfläche deutlich wahrnehmbare, mit ihm fest verbundene Marken trägt. An der Verschiebung dieser Marken gegeneinander ist dann eine vollzogene Verschiebung einzelner Plasmapartien gegeneinander festzustellen: Die Gesamtheit jener deutlichen, jedem einzelnen Ort der Oberfläche fest zugeordneten Marken ist das Silberliniensystem. Es macht alle Veränderungen der Oberfläche, an die es ortsfest gebunden ist, mit und zeigt so durch seine Gestaltsveränderungen Veränderungen in der Lage der Orte der Oberfläche an. Es ist ein Anzeiger, ein Indikator auch für diese rein räumlichen Veränderungen (denn daß es alle anderen ebenfalls anzeigt, wurde schon früher festgestellt, vergl. KLEIN, 1937), wie es einen anderen gleich zuverlässigen und empfindlichen nicht gibt. Daraus geht auch hervor, warum dieser Untersuchung gerade Silberpräparate zugrunde gelegt sind.

Wenn nun jetzt die, während der Entquellung möglichen, Entformungen in der Absicht durchgegangen werden, sie nicht nur morphologisch zu werten, sondern auch die Bedingungen kennen zu lernen, die sie bestimmen, so ist vorerst einmal festzustellen, was erfüllt sein muß, wenn die Form erhalten bleibt. Sobald diese Bedingungen bekannt sind, ergibt sich die Möglichkeit zu sagen, ob im Fehlen einer oder mehrerer derselben schon die gesuchten Entformungsursachen liegen können, bzw. ob und was außerdem in gewissen Fällen noch notwendig sein könnte. Die Bedingungen für

die Formerhaltung führen so auf die Spur der Entformungsbedingungen.

Wenn eine freie Infusorienzelle bei der Entquellung keine Entformung erfährt, so sind gewisse Bedingungen erfüllt, die zwei verschiedenen Bereichen angehören: Dem *äußeren* und dem *inneren* Bereich, oder anders, der Außen- bzw. Innenwelt, wie sich gleich ergeben wird. Zu den *äußeren* Bedingungen gehört der nötige freie Raum, das Tier darf nicht, durch diesen Raum beengende Körper, rein mechanisch aus seiner Form gebracht werden. Dies tritt z.B. dann ein, wenn viele Tiere beisammen sind, die sich bei fortschreitender Verdunstung des sie beherbergenden Wassertropfens an den Stellen längsten Wasservorhaltens zusammendrängen (Abb. 1), sich so gegenseitig den nötigen freien Raum wegnehmen und dadurch entsprechende Entformungen erleiden. Auch an Detritus und anderen festen Körpern, die durch kapillare Wirkung Orte längeren Wasservorhaltens sind und so die Tiere an sich ziehen, treten aus der angegebenen Ursache Entformungen auf.

Eine zweite, von außen kommende, Entformungsursache läge in chemischen oder anderen, von außen zugeführten Schädlichkeiten. Dieser Faktor scheidet hier aber aus, da im Vorliegenden nur die Entformungen in Betracht gezogen sind, die bei der Entquellung im normalen Lebensmedium, sozusagen „spontan“ und nicht nach experimentellem Eingriff auftreten. Im normalen Lebensmedium

und bei normaler Entquellung erreichen Salzkonzentrationen, Konzentrationsgefälle, Temperaturen u.a., obwohl sie unter Umständen (KLEIN, 1927) imstande sind das Silberliniensystem partiell oder total zu schädigen, nie eine die Form zerstörende Intensität, wenn sie auch manchmal eine durch *innere* Faktoren möglich gewordene partielle Entformung verwirklichen helfen (vergl. später). Dies fällt aber bereits in die nächste Sparte der Entformungsbedingungen.

Von dieser, die inneren Ursachen umfassenden Kategorie hängt vor allem die gleichmäßige Wasserabgabe über den ganzen Zell-Leib hin ab. Sowohl die einzelnen nebeneinander, als auch die einzelnen untereinander liegenden Partien bzw. Schichten müssen

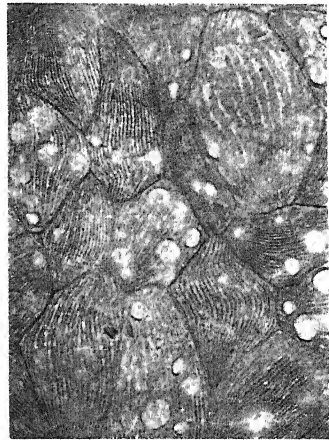


Abb. 1. *Colpidium campylum* und *Colpidium colpoda*. Silberpräparat. Entformung bei raumbehinderter Entquellung. Reichert Apochromat 8 mm, Comp. Ok. 6, Vergr.: 320.

dem verdunstenden Wasser gegenüber gleiche Durchlässigkeit aufweisen, müssen in dieser Beziehung sozusagen ein homogenes Ganzes bilden. Denn wenn eine Partie das Wasser länger hielte als die danebenliegende, dann entstünde ein Buckel, wenn andererseits einzelne Partien schneller das Wasser abgäben als ihre Umgebung, dann entstünden Dellen. Wenn Schichten, z.B. die Oberste, länger gequollen blieben als die das Wasser rascher abgebenden darunter liegenden Schichten, dann entstünden in dieser äußersten Schicht Runzeln bzw. Falten, ebenso wie dann, wenn die äußerste Schicht durch Wasserabgabe rascher starr würde als die Unterlage, so daß sie, wenn sie schließlich auch entquollen ist und sich verkleinert, keinen prallen Halt mehr gewährt und die Hülle über ihr faltig einsinken mußte, ähnlich der Schale eines vertrocknenden Apfels.

Als dritte innere Bedingung für Entformungen kommen aktive Gestaltsveränderungen der betreffenden Tiere während der Entquellung in Betracht.

Ob und in welchem Maße die angegebenen Möglichkeiten tatsächlich entquellende Ciliaten entformen können, werden die nun folgenden Fälle zeigen.

Ein Beispiel dafür, daß Mangel an nötigem Raum, also eine rein äußere Ursache, die betroffenen Tiere entformt, gibt die schon bezogene und besprochene Abb. 1. Alle Tiere die im Bereich dieser Ursache liegen, werden von ihr in gleicher Weise beeinflußt, es gibt hier, und das ist besonders hervorzuheben, weil für diesen rein von außen kommenden Einfluß bezeichnend, nicht jene individuellen Unterschiede, wie bei den in erster Linie oder ausschließlich von verschiedenen, beim gleichen Tier zeitlich wechselnden, inneren Zuständen ausgelösten Form- oder anderen Reaktionen. Es ist dies auch begreiflich, denn ein rein mechanisch beengter Raum zwingt eben den Tieren die angemessene Form auf, abgesehen davon welche verschiedenen inneren Zustände bei den einzelnen Tieren gerade vorliegen.

Die eben belanglos gewesenen inneren Zustände kommen in den Entformungen der folgenden Fälle bemerkenswert zur Geltung. Im allgemeinen sind, durch innere, individuell jeweils verschiedene, Zustände bedingte Veränderungen dann anzunehmen, wenn eng benachbart gelegene Tiere aus der Entquellung, im Gegensatz zu der diesbezüglichen Gleichmäßigkeit des vorigen Falles, ganz *verschieden* hervorgehen, Verschiedenheiten aufweisen, die bei der verhältnismäßigen Gleichheit (KLEIN, 1937) der äußeren Bedingungen nur durch Ungleichheit der inneren Zustände einzelner Tiere ermöglicht erscheinen: Die gleiche äußere Ursache trifft eben nicht bei jedem Tier auf die gleiche innere Situation, sei es im allgemeinen

oder im einzelnen und die Ungleichheit in dieser Beziehung zeitigt die Ungleichheit des Ergebnisses, das so einen diesbezüglichen Indikator abgibt.

Einen Formschaden, der in einfacher Art auf ortsweise Zustandsverschiedenheiten des Plasma's, also auf innere Ursachen hinweist, gibt Abb. 2a wieder. Die, das abgebildete Tier nächst umgebenden Tiere, von denen am Bild nur einzelne Partien zu sehen sind, wiesen weder Formschäden noch Schäden des Silberliniensystems auf. Die äußeren Bedingungen auf dem kleinen Feld, in dessen Mittelpunkt etwa das zu besprechende Tier liegt, zeitigten bei allen Tieren der Umgebung das gleiche Ergebnis, wie bereits gesagt, gute Erhaltung der Form und des Silberliniensystems. Der gleiche äußere Faktor traf bei allen diesen Tieren auf gleiche innere Faktoren. Nur im abgebildeten Tier waren diese teilweise anders (dort wo Zerstörungen vorliegen). Worauf diese örtliche Verschiedenheit zurückgehen kann, ist, nach Form und Lage der Schäden, nicht schwer zu erraten: Die Nahrungsvakuolen der Tiere bedingen auf Grund ihres besonderen, jeweils verschiedenen Verdauungsstufen entsprechenden Inhaltes, sehr oft verschiedene Veränderungen des Zustandes des sie umgebenden Plasma's, die so zum Ausdruck kommen, daß sich das darüberliegende Silberliniensystem entweder schlecht oder gar nicht mit Silber imprägniert oder überhaupt zerfällt oder schließlich Silber reichlich in Vakuole und Umgebung körnig niedergeschlagen wird. Die Verschiedenheit dieser Reaktion zeigt deutlich an, daß die Vakuolen jeweils ganz verschiedene Inhaltszustände aufweisen, die während der Entquellung gegebenenfalls auch den Zustand des umgebenden Plasma's beeinflussen, wie dies eben bei Fehlprägnierung oder Zerstörung des Silberliniensystems zum Ausdruck kommt. Ist der Einfluß eines bestimmten Vakuoleninhaltes so stark, daß während der Entquellung nicht nur das Silberliniensystem zerstört wird, sondern in geringerem oder größerem Ausmaß auch das umgebende Plasma angegriffen und zerstört wird, so ergibt sich der in Abb. 2a gezeigte Fall, der statt zweier Vakuolen zwei tiefe Krater mit ausgefressenen Rändern aufweist, in denen außer dem Silberliniensystem auch die Unversehrtheit der Form fehlt.

Läßt der eben geschilderte Fall ziemlich eindeutig die Art der von innen kommenden Entformungs-Ursache erkennen, so ist dies beim nächsten Fall (Abb. 2b) schon nicht mehr so leicht. Statt Krater zeigt das Tier eine mächtige Auftreibung, die bei fortschreitender Entquellung in einer Falte einsank. Die Entformung dieses Tieres, seine Auftreibung, verhält sich zu den Löchern des Vorigen gerade entgegengesetzt, sozusagen wie eine Positiv zum Negativ.

Die Ursache der vorliegenden Entformung ist nur insoferne eindeutig, als es sich dabei um innere Vorgänge handeln muß, denn die äußeren Bedingungen lieferten bei den Tieren der nächsten Umgebung, ebenso wie im vorigen Fall, durchaus formnormale Tiere. Die wirksam gewesenen inneren Vorgänge scheinen nicht einheitlich gewesen zu sein: Neben ungleichmäßiger Quellung bzw.

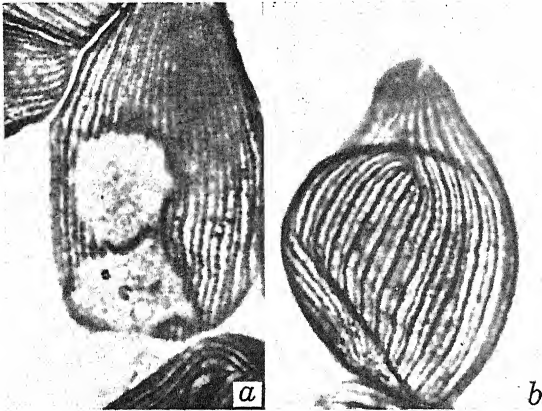


Abb. 2. a. Kraterbildung bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950. b. Beulenbildung bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950.

Entquellung des Plasma's dürfte auch eine aktive Formänderung, wie sie spätere Beispiele zeigen werden, mitbeteiligt gewesen sein. Der vorliegende Fall vereinigt verschiedene innere Entformungsmöglichkeiten, die im Folgenden noch für sich gezeigt werden, wodurch die hier vorliegende zusammengesetzte Ursache in ihre einzelnen Komponenten zerlegt werden wird.

Eine dieser Komponenten zeigt Abb. 3a. Ein Körperabschnitt des vorliegenden Tieres und zwar sein hinterer Pol, ist eingesunken. Er hat sich der Entquellung gegenüber anders verhalten als der übrige Zell-Leib. Er hat sich so verhalten, wie sich ein mehr Wasser enthaltender und dieses Wasser rascher abgebender Teil einem wasserärmeren, das wenige Wasser aber länger festhaltenden Teil gegenüber verhalten muß: Der stärker verwässerte Teil fällt ein, weil er seine stützende Unterlage und damit seine Form verliert, während der wasserärmere und festsubstanzreichere Teil seinen Unterbau und damit seine Form behält. Die vorliegenden Verhältnisse zeigen, daß bei einzelnen Tieren bzw. zeitweilig bei den Tieren überhaupt, der Wasserreichtum des Plasma's regionär verschieden sein kann. Tiere, bei denen dies gerade während der Entquellung der Fall ist, zeigen dann die entsprechende Entformung, während die anderen, in dieser Beziehung keine Verschiedenheiten aufweisenden Tiere formgerecht entquellen, so daß eng nebeneinander liegende Tiere, trotz der gleichen äußeren Bedingungen, eben auf Grund der inneren Verschiedenheiten, verschieden aus der Entquellung hervorgehen können.

Das Silberliniensystem ist über den ganzen Körper hin ungeschädigt, also in positivem Strukturzustand.

Das nächste Bild, Abb. 3b (*Colpidium campylum* Stokes), gibt ein Tier wieder, das eine ähnliche Entformung zeigt wie das vorrige Tier, nur findet sich das ganze Silberliniensystem in einem viel schlechterem Strukturzustand: Es ist knapp vor dem Zerfall und zwar sowohl auf dem entformten als auch auf dem nicht entformten Körperabschnitt. Die zur Entformung führenden Bedingungen sind also hier nicht verantwortlich für den beginnenden Strukturzerfall des Silberliniensystems, sonst müßte dieser Zerfall auf dem nicht entformten Körperteil fehlen. Der Zerfall muß eine das Tier als Ganzes treffende Ursache haben, die von innen oder von außen gekommen sein mag (KLEIN, 1937).

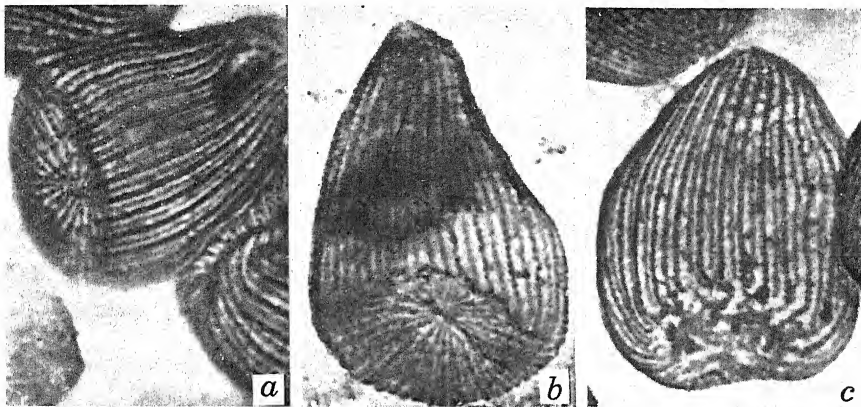


Abb. 3. a, b, c. Eindellungen bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950.

Bei einem weiteren Fall, Abb. 3c (*Colpidium campylum* Stokes), zeigt sich diesbezüglich wieder etwas anderes. In der nicht sehr großen Entformungszone, dem eingedellten hinteren Pol, zeigt das Silberliniensystem eine deutliche Reaktion und zwar struktureller und formativer Art, d.h. das System ist einerseits zerfallen und verballt, andererseits liegen aber auch, hauptsächlich am Rande gegen den nicht entformten vorderen Körperteil, formative Bildungsprozesse vor. Da das Silberliniensystem der nicht entformten, größeren vorderen Körperpartie weder strukturelle noch formative Reaktionen aufweist, so ist hier, im Gegensatz zu dem vorigen Fall, die Ursache der Entformung mit derjenigen der Reaktion am Silberliniensystem irgendwie gekoppelt oder gar gleich. Entformung und Veränderung des Silberliniensystems, die vorhin voneinander unabhängig waren, stehen hier eindeutig zueinander in Beziehung. Diese

beiden Fälle zeigen, wie verschieden bzw. gegensätzlich eine und dieselbe Voraussetzung, hier eine gleichartige Entformung, auf ein und denselben Faktor, das Silberliniensystem, wirken, so daß, da oft noch weitere Faktoren in ähnlicher Weise angekoppelt sind, eine verwirrende Mannigfaltigkeit des Ergebnisses möglich ist.

Wenn Entquellungsunterschiede nicht so, wie bei den drei letzten Fällen, in verschiedenen Körperabschnitten auftreten, sondern verschiedene Körper-Schichten betreffen, so ergibt sich wieder ein neuer Entformungstyp. Es treten Faltungen auf. Die Pellikula bzw. das Ektoplasma sind hier gegen das Entoplasma wasserärmer, verfestigen sich während der Entquellung bereits zu einer Zeit, da das Entoplasma noch viel Wasser enthält, soweit, daß sie sich nicht mehr in sich, d.h. ihre Gesamtfläche, verkleinern können, sondern, wenn das Entoplasma nach völliger Wasserabgabe zuviel von seinem Volumen verloren hat, über diesem zu klein gewordenem Kern als zu groß gebliebene Schale sich einfallen. Verschieden ist der Einfluß, von außen an das betreffende Tier anstossender Körper, auf solche Faltungen. Während das auf Abb. 4 wiedergegebene Tier durch den von rechts auf dasselbe stossenden Vorderteil eines zweiten Tieres in seiner Faltenbildung gar nicht beeinflußt wird, zeigt Abb. 5 daß die hier vorliegende Faltung von der, rechts dem Tier anliegenden, Flagellatenzelle geradezu ausgelöst wird: Es entsteht der Eindruck, als wollte sich das Infusor durch die Einfaltung vor dem anliegenden Körper zurückziehen. Wieder ein Beispiel dafür, wie verschieden ein und derselbe Faktor als Auslösung einer bestimmten Reaktion wirken kann.

Außer der Faltung tritt noch der Umschlag auf. Abb. 6 zeigt einen solchen bei *Chilodon uncinatus* Ehrbg. Der hintere Körperabschnitt ist von dorsal auf die ventrale Körperfläche umgeschlagen und zwar in einem Ausmaß, das als äußere Ursache nur eine äußerst kräftige Strömung im Wasser des eintrocknenden Tropfens haben könnte. Da solche Strömungen in diesem Bereich völlig ausgeschlossen sind, und, wenn sie künstlich erzeugt würden, noch eine ganze Reihe anderer Wirkungen haben müßten, ist die Ursache dieser Umschlagung vorerst rätselhaft. Da aber spätere Beispiele zeigen werden, daß aktive Formänderungen der Tiere während der Entquellung nicht so selten auftreten, bleibt auch für diese kräftige Umschlagung nur diese Ursache übrig: Das Tier verkleinert dadurch seine Oberfläche, es stellt der fortschreitenden Wasserabgabe eine Schutzmaßnahme entgegen (KLEIN, 1928, S. 185), es deckt sich gewissermaßen mit seinem eigenem Körper zu.

Daß auf die Kugelform abzielende Umformungen aktive Formänderungen als Ursache haben, zeigen die vor der Encystierung



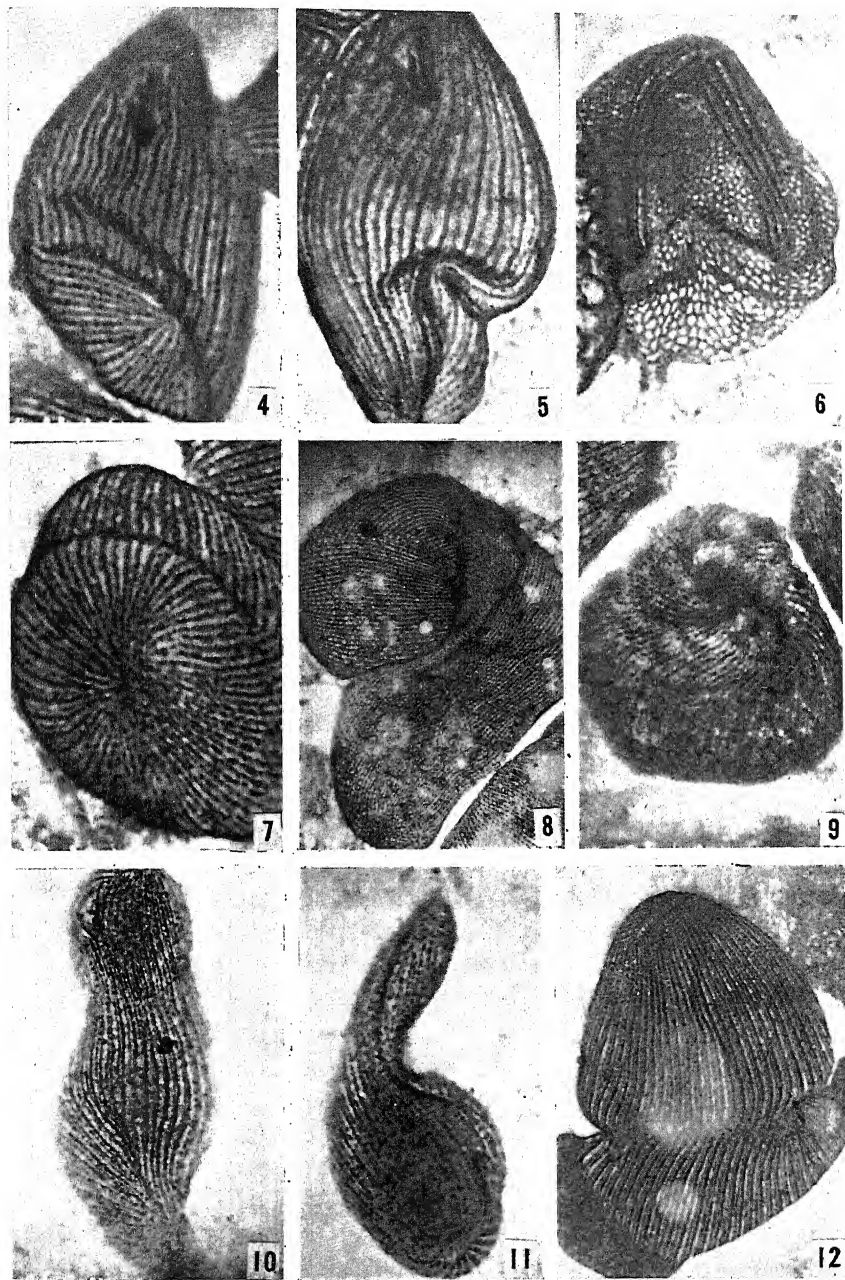


Abb. 4 u. 5. Faltungen bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950. Abb. 6. Umschlagung bei *Chilodon uncinatus*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950. Abb. 7. Einknickung bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950. Abb. 8. Einknickung bei *Paramaecium*. Silberpräparat. Reichert Apochromat 8 mm, Comp.-Ok. 3, Vergr.: 320. Abb. 9. Abkugelung mit schraubiger Verdrehung

an Ciliaten ablaufenden Vorgänge (KLEIN, 1929, S. 185 u.f.). Da die Fähigkeit aktiver Formänderung gegeben ist, erscheint es nicht verwunderlich, daß auch während der Entquellung Entformungen auftreten können, die als Ursache diese Fähigkeit haben. Beispiele solcher, während der Entquellung entstehender, nicht nur auf Oberflächenverkleinerung, sondern geradezu auf die Kugelform zielender Entformungen geben die Abb. 7, 8 und 9.

Die Verhältnisse auf Abb. 7 (*Colpidium campylum* Stokes) lehnen sich noch an die Abb. 6 an. Es ist ein Umschlagen der einen Körperhälfte auf die Andere. Abb. 8 zeigt bei *Paramaecium* ein Umbiegen der beiden Körperhälften gegeneinander, so wie dies auch vor der Encystierung zu beobachten ist (KLEIN, p. 185, 1929). Abb. 9 endlich zeigt an *Colpidium campylum*, daß die Kugelform auch durch schraubige Verdrehung des Körpers (KLEIN, 1929) erreicht werden kann.

Wieder ein anderer durch aktive Formänderung zustande kommender Entformungstyp ist auf Abb. 10 und 11 (*Colpidium campylum*) wiedergegeben. Diese Entformung zielt nicht auf die Kugelform, sondern auf Streckung und Windung des Körpers. Abkuglung verzögert die Wasserabgabe. Ein sich abkugelndes Tier leistet durch diese Umformung seines Körpers der Austrocknung Widerstand, wenigstens für kurze Zeit. Die Kugelform ist in Absicht auf die Austrocknung eine Widerstandsform. Das Tier versucht durch diese Umformung der Austrocknung zu widerstehen. Die beiden auf Abb. 10 und 11 abgebildeten Tiere hingegen machen einen entgegengesetzten Eindruck. Die Bewegtheit ihrer Formen deutet nicht auf ausharrenden Widerstand, sondern auf den Versuch zur Flucht. Herauszuwinden versuchten sich die Tiere aus der Gefahr, nachdem Davonschwimmen wegen zu starker Verknappung des Wassers nicht mehr möglich war. Im Gegensatz zur Widerstandsform lägen hier somit Fluchtformen vor, beide durch aktive Formänderung möglich. Da die Entquellung auf lebendige Tiere mit voller Reaktionsfähigkeit trifft, so ist es nicht verwunderlich, wenn die Tiere auf die einsetzende Schädlichkeit des immer größer werdenden Wassermangels, den vorhandenen Fähigkeiten entsprechend, reagieren. Es ist nur merkwürdig, daß die Tiere individuell verschieden, einmal diese und dann wieder eine andere „Handlung“ der Schädlichkeit entgegenstellen, z.B. wie hier, einmal

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bei *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr. 950. Abb. 10. Fluchtform von *Colpidium campylum*. Silberpräparat. Reichert Achromat 7, Plan-Ok. 3, Vergr.: 950. Abb. 11. Dasselbe. Abb. 12. *Colpidium colpoda*. Silberpräparat. Abschnürung einer zerfließenden Körperpartie. Reichert Apochromat 8mm, Comp. Ok. 8, Vergr.: 450.

Widerstands- dann, in der gleichen Situation, Flucht-Versuch, beides verschiedene Schutzmaßnahmen gegen die gleiche Schädlichkeit.

Der letzte Fall (Abb. 12, *Colpidium colpoda*) zeigt eine Schutzmaßnahme eigener Art. Das Tier hat während der Entquellung einen Plasmaschaden erlitten, das Plasma zerfließt unten seitlich, das Tier rinnt nach dieser Seite aus. Durch aktive Formänderung versucht das Tier sich durch eine subäquatoriale Einschnürung gegen die zerfließende Partie abzugrenzen, sozusagen eine Demarkationslinie zu bilden. Zu welchem Erfolg dieser Versuch geführt hätte, läßt sich nicht sagen, da er, durch die inzwischen vollzogene Entquellung nicht zu Ende kommen konnte. Die Raschheit der Reaktion kann man sich vorstellen, wenn man bedenkt, daß ein Plasmaschaden erst bei recht fortgeschrittener Entquellung, wenn sie als diesbezügliche Schädlichkeit wirken kann, entsteht und dann bis zur vollendeten Entquellung nur mehr sehr wenig Zeit, höchstens wenige Sekunden zur Verfügung stehen.

Die Entquellung wird also von den Tieren nicht nur passiv hingenommen, sie können vielmehr dann, wenn eine etwas zu lange Entquellungszeit Schäden verursacht, oder überhaupt Zeit zu diesbezüglichen Reaktionen bleibt, durch aktive Formänderungen Schutzmaßnahmen dieser Schädlichkeit entgegensetzen.

Zum Schluß sei noch erwähnt, daß die vorgeführten Entformungen im allgemeinen nicht häufig sind. Zeitweise trifft man sie in einer Kultur häufiger, zeitweise fehlen sie ganz, so daß es den Anschein hat, als wären die Tiere in einer bestimmten Phase ihres Einzel- oder „Kultur“-Lebens für Entformungen besonders anfällig.

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## Failure of Cytokinesis During Microsporogenesis in *Zea mays* Following Heat Treatment <sup>1)</sup>

By

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Beadle (1932) described a failure of cytokinesis in maize during the meiotic divisions in some of the microsporocytes and the inheritance of the abnormality as a simple recessive character. The failure of cytoplasmic division was accompanied by aberrant chromosomal behavior which was followed by the degeneration of the 2n and 4n cells. Only microspores and pollen grains of normal size were observed and no large cells with metabolic nuclei of normal appearance were present.

The present paper is concerned with the hereditary failure of cytokinesis during premeiotic and meiotic divisions in some of the cells during microsporogenesis. The divisions of the nuclei during this stage were entirely normal, resulting in the formation of binucleate or multinucleate spores when there was a failure of cytokinesis.

**Material and Methods.** The culture originated in a heat treatment experiment to induce chromosome doubling (Randolph, 1932). The plants were selfed for four generations after the original heat treatment, which was applied to the young embryos obtained from a cross between two normal inbred strains that did not show either male or female sterility. Out of 38 normal diploid sister plants grown from treated seeds and examined at the pollen shedding stage, 37 were found to be normal, while one plant, that originated the culture under discussion, showed about 50% of aborted pollen and had the ear approximately half filled. This partial sterility was transmitted to the three succeeding generations. During the summer of 1938 two plants survived out of a dozen kernels

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The writer wishes to express his thanks and appreciation to Doctors R. A. Emerson, L. W. Sharp, and L. F. Randolph for valuable suggestions and criticism, and to Dr. Randolph also for his generosity in providing material for this study.

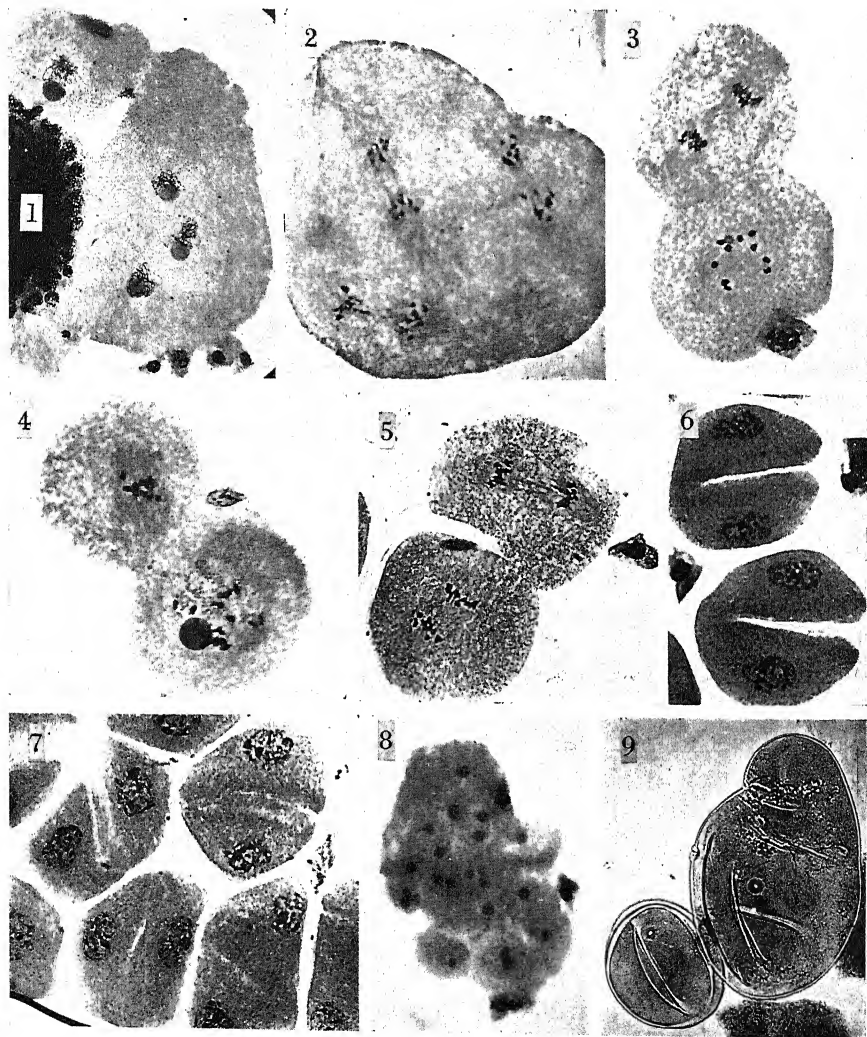
planted and were open pollinated. The ears on these plants were very small and had only a few kernels. The young tassels were preserved in acetic alcohol and sporocytes were examined after acetocarmine treatment.

**Description.** Among the normal microsporocytes and microspores it was found in many cases that several cells were either completely or partially united, forming plasmodial masses (fig. 8). In other cases relatively few nuclei were involved in such plasmodial masses (fig. 1-2). These masses were either spherical or ellipsoidal in shape, or in the form of a chain of cells which were either completely or partially united (fig. 11-12). Often there was no sign of the formation of any partition between the two or more nuclei lying in a common mass of cytoplasm (fig. 1-2). In other instances the united cells were separated from each other by a very thin layer of a cytoplasm or by a cell-plate, traces of which may be seen in figures 3-4. When two or more cells were partially united a cell wall formed between them except at the region of attachment when such a wall was absent (fig. 5, 11-12). The region of attachment may be either large or small, and the adjacent cells may be separated by either a plasma membrane or a cell-plate.

The nuclei in these united cells were usually in the same stage of division (fig. 1-2, 5, 11), as is usual in normal stocks of maize in which adjacent cells ordinarily are in the same stage of development. In certain instances, however, it was found that in two or more partially or completely united cells the nuclei were in different stages of division (fig. 3-4). Otherwise the first meiotic division in both normal and united sporocytes seems to be quite normal. The pachytene threads appear to be normal, with the exception of a loop formation (loose pairing) of one chromosome observed in several cells. At diakinesis ten pairs of chromosome can be seen. Metaphase and anaphase usually are normal, although lagging of one chromosome often was observed. This, together with the loop formation at the pachytene stage, may be the result of inversion in one chromosome. No abnormality of any kind in spindle formation nor any incorporation of two or more nuclei into a common nucleus was observed in the united cells.

The condition of the plasmodial masses and the partially united cells does not give any indication as to the cause of such a cell union. However, studies of cytokinesis at the end of both meiotic divisions indicate that these plasmodial masses and partially united cells must have resulted from the failure of cytokinesis during the premeiotic mitoses.

**Abortive cytokinesis during meiosis.** In maize microsporo-  
cytes cytokinesis takes place after both the first and the second  
meiotic divisions and is essentially similar in both cases. At the  
end of each telophase the spindle fibers appear to shorten and thicken  
at the equator; formation of a cell-plate in this region follows.



Figs. 1-9. 1. Four connected sporocytes, three of which are in a common cytoplasm, the result of a complete and partial failure of cytokinesis during the last two premeiotic divisions.  $\times 135$ . 2. Three sporocytes in the first anaphase lying in a common cytoplasm.  $\times 257$ . 3, 4, 5. Two connected sporocytes in the first division, the result of partial failure of cytokinesis during the last premeiotic division.  $\times 257$ . 6, 7. Partial failure of cytokinesis during the first division.  $\times 257$ . 8. Multi-nucleate plasmodial mass of young spores. Traces of a partition wall can be seen between some of these nuclei.  $\times 135$ . 9. Hexanucleate and normal spore with a heavy wall developed.  $\times$  about 135.

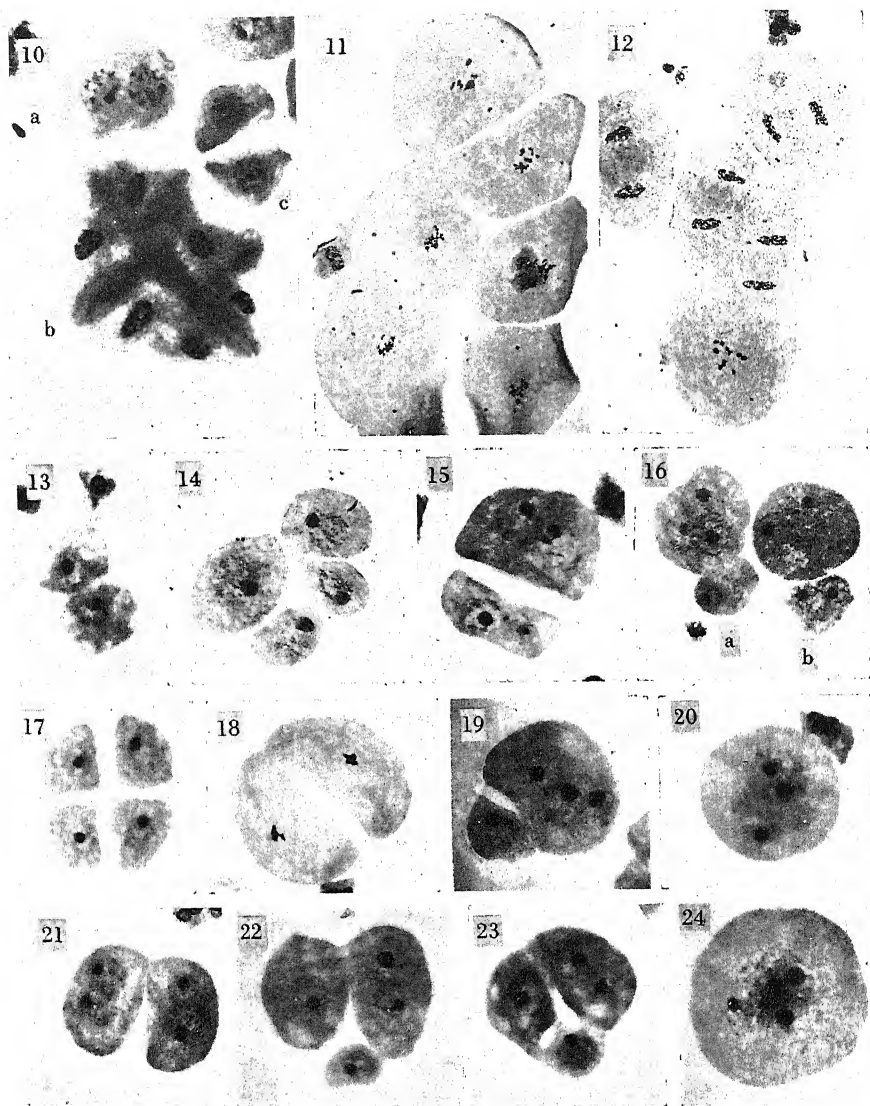
Only then do fibers begin to appear just beyond the periphery of the plate, which proceeds to increase in diameter. Then the spindle fibers begin to disappear and the plate begins to transform into the partition wall (Reeves, 1928). At about the time the cell plate is definitely formed, the chromatin of each daughter nucleus loosens up and nuclear membrane is formed around it.

In the sporocytes of the plants under discussion cytokinesis at the end of the first meiotic division may take place normally or it may fail partially or completely. The failure of cytokinesis is apparently due either to the failure of cell-plate formation, or to the failure of the plate already formed to continue its transformation and allow a separation of the daughter cells. In a number of figures it was observed that while the two daughter nuclei already had reached the metabolic stage and had acquired nuclear membranes there was no sign of any alteration of the spindle fibers at the equator, this resulting in a binucleate cell (fig. 10a). In other instances alteration was apparent but there was no sign of actual cell-plate formation when normally it should be occurring (fig. 10b). In still other instances it was found that at the end of the first meiotic division the cell-plate had formed but had failed to split or allow cytokinesis to occur throughout the whole equator, this resulting in the continued attachment of the two daughter cells at different points in the equatorial region. Figure 6 illustrates the failure of the cell-plate to form or to split in one region of the equator, while in figure 7 are shown cells in two of which this failure occurred in two peripheral regions.

The failure of cytokinesis after the first meiotic division may be preceded by the failure of cytoplasmic divisions during premeiosis. Figure 10b shows six nuclei in a mass of cytoplasm which has failed to complete its division. The surrounding cells are completing the first meiotic division, which clearly indicates that the six-nucleate mass is a result of the failure of cytokinesis during the last two premeiotic and the first meiotic division. The presence of six nuclei instead of the expected eight is probably due to the fact that during the last premeiotic division cytokinesis was partially successful, so that only three out of four cells remained connected. A comparison of figures 2 and 10b will clarify this point. Other observations indicated that such failure of cytokinesis during the premeiotic and the first meiotic divisions may be either complete or partial.

Cytokinesis also may fail completely or partially during the second meiotic division. When this occurs, and if the cytoplasmic division was normal during all the preceding divisions, binucleate spores are formed. In case of partial failure of cytokinesis binu-





Figs. 10-24. 10a. Complete failure of cytokinesis during the first meiotic division. b. The result of failure of cytokinesis during the premeiotic and first meiotic division. c. Two normal daughter cells after the first meiotic division.  $\times 180$ . 11, 12. Chain of sporocytes complete or partially connected.  $\times 120$ . 13-16. Binucleate spores resulting from either partial or complete failure of cytokinesis during the second meiotic division.  $\times 180$ . 17. A quartet of young normal microspores.  $\times 180$ . 18. Partial failure of cytokinesis during the first meiotic division. Two connected sporocytes at second metaphase.  $\times 180$ . 19-23. Quadrinucleate spores of various shapes resulting from either complete or partial failure of cytokinesis during both meiotic divisions.  $\times 180$ . 24. A quadrinucleate spore resulting from a complete failure of cytokinesis during both meioses.  $\times 180$ .



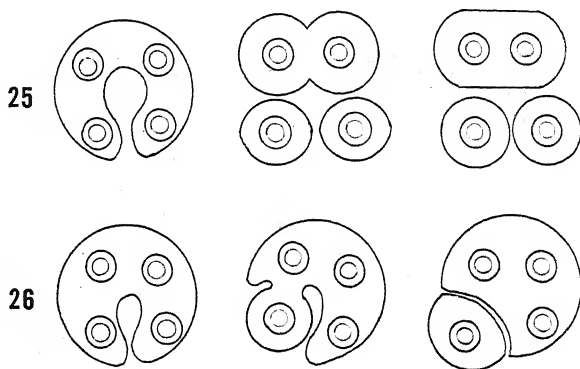
cleate spores are irregular in size and shape (fig. 13-14). When the failure is complete the young spores are characteristically oblong in shape (fig. 15) and may later become spherical (fig. 16b). It is noteworthy that in this and in many other cases spores were of unequal size because of the uneven distribution of cytoplasm among the daughter cells, especially where partial failure of cytokinesis occurred. A quartet of normal young spores is shown in figure 17.

Binucleate spores probably would also develop if cytoplasmic division failed during the first meiotic division only, although no satisfactory figures are available which would indicate this. Let us assume that two partially connected cells in the metaphase of the second meiotic division (fig. 18) would successfully complete the second division. Then two uninucleate and one binucleate spore would develop as

illustrated in the figure 25. It seems probable that the failure of cytokinesis at the first meiotic division is usually accompanied by at least a partial failure during the second meiosis. Many spores found to have three nuclei (fig. 19-20) probably resulted from a partial failure of cytokine-

sis during both meiotic divisions as shown in figure 26. The partial failure of cytokinesis during both divisions may also result in the development of quadrinucleate spores of various shapes, as is shown in figures 16a, 21-23. These illustrations are self explanatory and need no detailed description. Figure 21, for example, illustrates partial failure of cytokinesis during the first meiotic division and complete failure during the second. Figure 23 illustrates partial failure of cytokinesis during first division, while during the second division cytokinesis completely failed in one daughter cell and partially failed in the other.

When cytokinesis fails completely during both meiotic divisions quadrinucleate spores are formed (fig. 24). Such spores are usually



Figs. 25-26. 25. Diagram of the formation of a binucleate spore resulting from partial failure of cytokinesis only at the first meiotic division. 26. Diagram of the formation of a spore with three nuclei due to a partial failure of cytokinesis at both meiotic divisions.

symmetrical in shape and under a cover glass assume the form of a disk. They are considerably larger than uni- and binucleate spores. Microspores containing from five to eight or more nuclei (fig. 8-9) have been found, and these undoubtedly resulted from cells in which cytokinesis failed during premeiotic and both of the meiotic divisions.

Multinucleate spores develop a heavy wall just as normal spores do (fig. 9). However, plasmodial masses having more than eight nuclei apparently fail to develop a spore wall. The frequency of multinucleate spores varies from flower to flower even on the same plant; it may be as low as 5-10% and as high as 60-80%.

Partial or complete failure of cytokinesis during premeiotic and meiotic divisions seems to have no effect on the process of nuclear division. Except for lagging and an occasional elimination of one chromosome, the nuclear divisions appear to be normal, and the daughter nuclei pass thru metabolic stages which are normal in appearance.

**Size of microspores.** It is a well known fact that in many species of plants the size of the cells and the pollen grains in a polyploid series shows a positive correlation with chromosome number. Randolph (1935) has demonstrated that in maize  $2n$  pollen grains are considerably larger than  $1n$  grains. The same correlation was found in multinucleate spores in this study. Measurements were made of normal ( $1n$ ), binucleate and quadrinucleate spores. The spores tend to become flattened under a cover glass, and only those which were circular in outline or nearly so were measured. Several slides were used for this purpose, and in the case of nearly spherical spores, the larger diameter was measured. Although it cannot be claimed that large cells and small cells were

Table 1. Size frequency distribution of uninucleate, binucleate and quadrinucleate microspores

Microspores	Diameter in microns—lower class limit												Total	Mean	$\sigma$
	40	50	60	70	80	90	100	110	120	130	140				
Uninucleate	7	22	33	34	8								104	66.3	$10.54 \pm .4928$
Binucleate				8	19	28	23	20	7				105	99.7	$13.53 \pm .5391$
Quadrinucleate						9	12	30	14	6	2		73	115.3	$16.79 \pm .9875$

flattened in the same proportion, it was found that there is a definite correlation between the size of spores and the number of nuclei present in them (table 1). The binucleate spores were considerably larger than uninucleate ones, while the quadrinucleate spores were still larger. The mean diameter of the normal spores

was 66.3 microns, of the binucleate spores 99.7 microns, and that of quadrinucleate spores 115.3 microns. Therefore, it seems to be unessential, so far as the size of cells is concerned, whether the two chromosome sets are present in the same or in separate nuclei of the cell.

**Pollen sterility.** The preserved young tassels of the two plants under consideration contained in abundance of normal appearing spores, but no mature pollen grains. The gametophytic divisions were not observed. Therefore nothing definite can be said about pollen abortion, which was recorded in this line during four generations of selfing. However the condition of the spores offers some explanation for the partial male sterility. It is reasonable to assume that most of the multinucleate spores do not develop into functional pollen grains. Moreover, among the uninucleate spores in many cases it was found that the nucleolus broke up into several micronucleoli, and it is questionable whether such spores would develop into normal pollen grains. As pointed out by Beadle (1932), it is quite possible that conditions which bring about failure of cytokinesis, although they may not be obvious during meiosis in some cells, nevertheless may result in pollen sterility. Finally, lagging and elimination of one chromosome during meiotic divisions observed in these two plants may also result in pollen sterility. It is obvious, however, that the failure of cytokinesis and the lagging of one chromosome are two independent phenomena.

**Comments.** Cytoplasmic division has been observed occasionally to fail after nuclear division in many organisms. It is also a well known fact that the frequency of such failures is increased after many different treatments (see Sharp (1934) for a general discussion, and Goodspeed and Uber (1939) for the effects of radiation). Blakeslee (1930) and Beadle (1932) reported recessive genes in *Datura* and maize respectively, which brought about the failure of cytokinesis. The failure of cell division reported here is also probably hereditary, and originated after exposure of the young embryo to the effect of high temperature. The exact genetical nature of this abnormality is a subject for further studies.

### Summary

A case of failure of cytokinesis in the anthers of maize during premeiotic and meiotic divisions is described. The failure of cytoplasmic division has no evident effect on the course of the nuclear division. The microsporocytes in which the cytoplasm fails to

divide develop into multinucleate spores. The size of these spores is roughly proportional to the number of nuclei present.

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## Further Studies on Sex Linked Chromosome Abnormalities

By

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The author has expressed the opinion that so-called sex chromosomes are meiotic abnormalities induced by previous hybridization of the species, and that these abnormalities have been encouraged to persist over a number of generations through the advent of the dioecious condition in an otherwise monoecious or perfect flowering genus. That infers that the so-called sex chromosomes are the product of a disturbance in the meiotic process, hybridization, and the peculiar circumstances attendant upon the development of unisexuality. Investigations of plants exhibiting a wide variety of cytological and genetical conditions is necessary to thoroughly establish such a contention. In fact, criticism of the conventional theory of sex chromosomes has repeatedly pointed to errors whose stimulation has been the restricted source of evidence upon which the theory was based. To avoid similar embarrassment as well as to continue the construction of the ideas as set forth in a recent paper on the origin of so-called sex chromosomes in the Angiosperms, Jensen 1939, the author submits a study of microsporogenesis in three species, namely; *Xanthorrhiza apiifolia* L'Her, *Chamaelirium luteum* L., and *Smilax herbacea* L. Since each was studied for a particular reason, theoretical considerations will be included with the descriptive material under the heading of each species.

Material for these investigations was collected in the Swannanoa valley of western North Carolina, was preserved in modifications of Carnoy's fluid, was treated and imbedded according to the Jeffrey technique (Jeffrey 1928) and stained with Heidenhain's iron-alum haematoxylin, staining and decoloring slowly to obtain maximum differentiation. Zeiss apparatus was used in the microscopy.

*Xanthorrhiza apiifolia* L'Her. As the author has previously indicated, Jensen 1939, the cytological condition of monotypic, unisexual species is important to the argument in that recent hybridization of the species seems impossible and, if the author's opinion be correct, no evidence of so-called sex chromosomes should be found. *Xanthorrhiza apiifolia* is monotypic and polygamo-dioecious. Some plants were found which apparently produced only flowers of one sex, although the mixed condition was more frequent. Because the

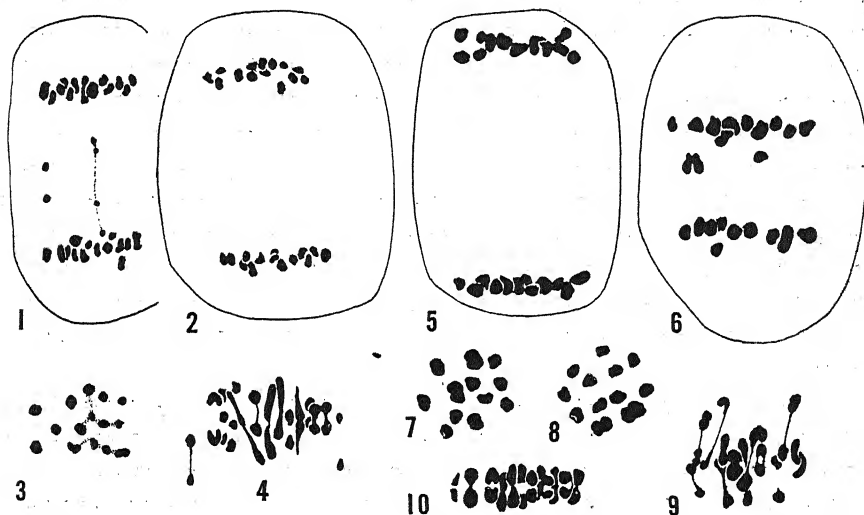
sexual condition of this species is evidently in a state of flux, presumably evolving into the strictly unisexual type, the behavior of its reproductive chromosomes should be of interest. Would there be some indication of sex chromosome evolution—, that is the question.

The haploid number of chromosome for this species appears to be fifteen, fig. 3, as seen from the polar view of the heterotypic metaphase. Some of the chromosomes appear to retain their old spireme connections up to this stage. Generally, however, they are distinct after the dissolution of the nuclear membrane. Fig. 4 shows this as seen in a side view of the same stage. Usually the metaphase configuration was flat, although a few chromosomes were occasionally a bit out of line. The early separation of some of the smaller chromosomes leads to an uneven figure during the late metaphase and early anaphase of the first division. With but one exception, shown in fig. 1, the chromosomes were found to move simultaneously toward the pole of the spindle. No evidence of heterochromosomes was discovered. As seen in fig. 4, the chromosomes appear to consist of identical units. No extruded chromatin was observed in the cytoplasm, nor were extra nuclei formed. The second meiotic division was quite regular and the pollen appeared to be structurally perfect.

Thus, *Xanthorrhiza apiifolia*, a monotypic species approximating the dioecious state, reveals no meiotic abnormality which could suggest the development of sex chromosomes. As was expected, no diagnostic hybrid characteristics were found in the meiotic process, and therefore no evidence of recent hybridization of the species. This compares with the conditions found in *Pyrularia pubera*, Jensen 1939, and in so far as it is an additional case, contributes to the argument that so-called sex chromosomes will only be found in species capable of recent hybridization. Though monotypic species may well be of hybrid origin, the absence of parental or related forms in the genus would certainly suggest that such an origin lay far in the past—, far enough so that whatever meiotic irregularities might once have existed have long since disappeared.

*Chamaelirium luteum* L. This is one of two species which comprise the genus *Chamaelirium*. In view of the author's findings in several native species of *Smilax*, Jensen 1937, which is a large genus of some 300 species (Engler-Diels 1936), it was thought advisable to investigate a small dioecious genus of the same family. The distinguishing features of the two species are so slight that there is every indication that they are closely related. Consequently, one might expect to find meiotic irregularities or evidence of heterochro-

mosomes. However, in microsporogenesis the first reproductive division proceeds with regularity. The haploid number of chromosomes appears to be twelve and composed of two duplicate sets of six each. Two large chromosomes stand out in contrast with the others, fig. 7 and fig. 8. The metaphase finds all the chromosomes gathered at the plate, fig. 10, and a bit irregularly dispersed in the early anaphase, fig. 9. At first there seemed to be several instances of heterochromosome formation. Closer study however, and comparison with other pollen mother cells revealed that all the chromosomes were composed of similar units. Occasionally a few chro-



Figs. 1-4. *Xanthorrhiza apiifolia*. 1. Only instance of laggards seen. 2. Heterotypic anaphase. 3. Polar view of heterotypic metaphase. 4. Early anaphase showing equal sets of chromosomes. Figs. 5-10. *Chamaelirium luteum*. 5. Heterotypic anaphase. 6. Middle anaphase showing odd laggards. 7 and 8. Polar view of anaphase complements in the first division. 9. Early heterotypic anaphase. 10. Heterotypic metaphase.

mosomes would lag on the spindle, fig. 6, but at no time were the laggards extruded into the cytoplasm and stranded there. All the chromosomes were included in the daughter nuclei, as checked with counts, and no extra nuclei were seen. The second division is quite normal and the pollen appears to be structurally perfect. Seed development depends upon fertilization because female plants which had been protected from pollen produced no seed. If the occurrence of an odd laggard during the heterotypic division can be credited with much significance, it is the sole evidence of previous hybridization of the species. However, the chromosome complement of the somatic cells seems to be tetraploid and possibly this may be one of those frequent cases of doubling the number and thereby eliminat-

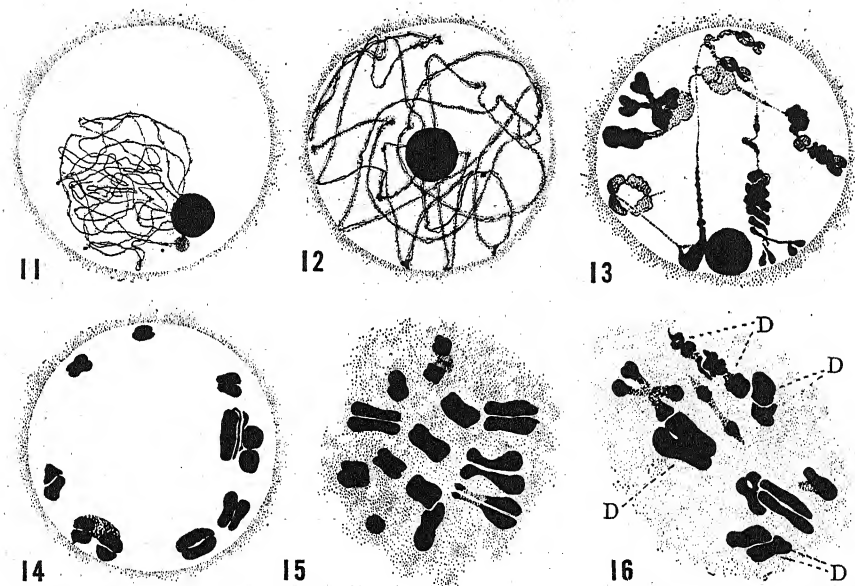
ing the disturbance in the meiotic process. However, this remains largely problematical. The fact remains that no evidence was found which might suggest that heterochromosomes or sex chromosomes were present in this species.

*Smilax herbacea* L. The author has already reported his study of microsporogenesis in *Smilax glauca* and *S. rotundifolia*, Jensen 1937. Although it belonged to another section of the genus, *S. herbacea* was just another species which ought to be looked at. Even though it does not compare with material of *Lilium*, *Trillium*, *Allium*, or *Tradescantia*; the carrion flower is a good subject for a study of the origin of meiotic chromosomes. Exact knowledge of what takes place before the reduction division is vital to a discussion of the theory of sex chromosomes. This theory depends upon the successful maintenance of chromosome individuality, which in turn theoretically depends upon the successful operation of synapsis or active pairing. Because observations on numerous species have given rise to the suspicion that the active pairing of chromosomes prior to the reduction division is far from a universal fact, the convenience of *S. herbacea* was utilized. A description of spireme and chromosome formation follows.

Two observations stood out over others when the synizetic stage of the spireme was studied. The first was the general occurrence of a second and smaller nucleolus attached to the original and principal nucleolus. The smaller nucleolus always faced the weft of contracted spireme, as shown in fig. 11. In fig. 12 the spireme had expanded once more and the strands were considerably thicker. However, there was a striking absence of free ends of chromatids. As was later revealed, the haploid chromosome count for this species seems to be thirteen. That would call for twenty six chromatids, and since each chromatid must have a minimum of two ends, fifty two ends should present themselves. Without question, the spireme in this stage is practically continuous. In whole nuclei only two or three free ends could be discovered and in most pollen mother cells none could be found. The doubleness of the spireme was partly discernible as early as the synizetic stage at which time there was also a dearth of free ends to the supposed chromatids. Prior to diakinesis, fig. 13, the chromosomes become distinctly segmented in the spireme into individual chromosomes or blocks of chromosomes. At this stage the spireme is clearly tetrapartite and separated into chains of chromosomes (that is, no longer continuous). The chromosome next to the nucleolus and the one at the top of the nucleus reveal the four parted nature of the twisted spireme. Fig. 14 is the conventional diakinetik figure and similar conditions



in other pollen mother cells led the author to believe that the haploid chromosome count for the species was nine. However, when the nuclear membrane disappeared, more than nine chromosomes were visible in the cytoplasm. Presumably eight chromosomes are associated in four groups of two each. Fig. 15 is a polar view of a heterotypic metaphase showing thirteen chromosomes, while fig. 16 is the same of a slightly earlier stage. The four double associations may be seen in the latter, and are marked with a D.



Figs. 11-16. *Smilax herbacea*. 11. Synizesis. 12. Continuous and double spireme. 13. Partially segmented spireme showing tetrapartite nature. 14. Diakinesis. 15. Polar view of heterotypic metaphase. 16. Early heterotypic metaphase. All stages taken from microsporogenesis.

The remainder of microsporogenesis proceeds with utmost regularity. Cytokinesis occurs after both divisions. Four microspores are formed and though completely mature pollen was not observed, grains which had obtained most of their growth gave no indication of abortion.

The author is aware of the following determinations of chromosome number in the genus *Smilax*.

<i>S. herbacea</i>	12	Humphrey	1914
"	12-13	Elkins	1914
"	13	Lindsay	1930
"	13	Jensen	1939
<i>S. hederacea</i>	15	Nakajima	1937
<i>S. Oldhami</i>	16	"	1937
<i>S. China</i>	15.	"	1937
<i>S. glauca</i>	14	Jensen	1937
<i>S. rotundifolia</i>	16	"	1937

Apparently there is no consistent basic chromosome number yet discernible among the determinations already made. However, the fact that there is considerable variation in the numbers would suggest that the genus is a good subject for investigation, particularly since there are some three hundred species.

Taken by itself, the significant point to be made from the study of *S. herbacea* is the absence of free chromatids during the prophase of meiosis. It is difficult to conceive how the spireme could overcome the engineering difficulties and pursue the act of pairing in a successful manner. The condition of the spireme in *S. herbacea* is basically similar to the conditions found in *S. glauca* and *Dioscorea quaternata*, Jensen 1937, *Rumex acetosa*, *R. acetosella* and *Rheum rhaponticum*, Jensen 1936, and *Ilex opaca* Jensen 1939. Though considerable work remains to be done on this moot point, enough evidence is forthcoming to warrant skepticism over the more conventional assumptions about the meiotic process. Careful manipulation of the staining technique and patient microscopy will often reveal the continuous nature of the spireme when more superficial methods of study would lead one to accept the presence of individual chromatids in the earlier premeiotic nuclear stages. If the spireme from which the meiotic chromosomes arise is fundamentally continuous, a more reasonable explanation would be to consider the doubleness to have arisen by means of a longitudinal splitting of the spireme. Of course, such a conclusion rather embarrasses the hypothesis of strict chromosome individuality; and therefore reflects upon the theory of sex chromosomes. This substantiates the position taken by Jeffrey and Haertl 1938 in regard to *Trillium grandiflorum* in which they cast definite doubt upon the entire synaptic arrangement. As a consequence, even if one were to find heterochromosomes in *S. herbacea*, and subsequently find that they fitted into a sex chromosome pattern, their function as determinants of sex would be out of the question in that the individuality of the chromosomes is probably lost somewhere before the initiation of the reduction division. The author to some degree appreciates how much nicer it would be to roll along with the structural mechanisms so vitally needed by the Weismann school of cyto-genetics. Nevertheless, impartial observations on the development of the meiotic chromosomes in this species makes another conclusion more in keeping with the facts.

### Summary

Meiosis in male plants of *Xanthorrhiza apiifolia* L'Her is strictly regular and gives no indication of any abnormality or

inequality among the chromosomes which might be interpreted as a sex chromosome complex. No diagnostic hybrid characteristics were found. This bears out the author's prediction and adds another case which is cited in support of the opinion that so-called sex chromosomes will not be found in monotypic species because recent hybridization has not been possible in such species. The chromosome number appears to be  $n=15$ .

Meiosis in *Chamaelirium luteum* L. is also quite normal, without any indication that a sex chromosome complex is present. Little evidence was found for recent hybridization of the species. The chromosome number was determined as  $n=12$ .

The origin of the meiotic chromosomes was studied in *Smilax herbacea* L., as a result of which the mechanism of active pairing or synapsis is questioned. The meiotic chromosomes seem to arise from a fundamentally continuous spireme which splits longitudinally and later becomes segmented into the haploid number of chromosomes. Eight of the meiotic chromosomes are associated during diakinesis in groups of two each. The chromosome number has been determined as  $n=13$ . Meiosis is normal with no trace of diagnostic hybrid characteristics.

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## Cyto-genetic Studies of *Drosophila montium* (Report I)

By

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### 1. Introduction

Since the end of 1937, I have devoted myself to genetical and cytological studies of *D. montium* under the direction of Prof. T. Komai. The results obtained so far are published in the present report. Before going further, it is my pleasure to record here my indebtedness to Prof. T. Komai for his kind guidance. I wish to express my hearty thanks also to Dr. M. Chino, Mr. S. Fujii and Mr. T. Takaku for their kindness in giving me valuable aids in many ways.

### 2. Material

*D. montium* resembles *D. melanogaster* in its general features, but can be distinguished from it in that the eye is considerably larger and the body is much slenderer, and especially that the sex-combs are found on both the first and second tarsal joints of the prothoracic leg. The species also resembles *D. auraria* very closely. The only marked difference consists in the presence in *D. auraria* and the absence in *D. montium* of snowy white sprinkling on the carina of male.

The species is widely distributed, very common in the tropical regions of the Eastern Hemisphere, and has been recorded from Africa, Formosa, Java, India, Samoa, Sumatra, etc. (De Meijere 1916, Duda 1924, 1926, Malloch 1934, Kikkawa & Peng 1938).

Kikkawa discriminated two races among the species inhabiting Japan according to the difference in chromosomes as mentioned in the next chapter.

### 3. Cytological findings

#### a. Gonial chromosomes

The oogonial chromosome group of *D. montium*, as stated by Kikkawa (1936), consists of four pairs of chromosomes, namely, two large V-shaped pairs, a rod-shaped pair and a pair of small chromosomes which are V-shaped in Race A and rod-shaped in Race B (Fig. 1, a, b).

In the spermatogonial group of Race B, one of the rod-shaped chromosomes is replaced by a small V-shaped chromosome (Fig. 1, e).

In the oogonal metaphase of the non-disjunctional female of Race B, a small V-shaped chromosome is always found in the neighbourhood of one pair of the rod-shaped chromosome (Fig. 1, d).



Fig. 1. Gonial chromosomes. ( $\times 2300$ ). a-d. Oogonal chromosomes. e. Spermatogonial chromosomes. a. A-race. b. B-race. c. Hybrid of A & B. d. XXY female e. B-race (X, X-chromosome. Y, Y-chromosome. h, heteromorphous chromosomes.)

These findings show that the small V is the Y-chromosome and the rod is the X-chromosome. The X-chromosome may be recognized from the other small chromosome by having two constrictions. The latter chromosome which is rod-shaped in Race B and V-shaped in Race A, has only one constriction, at about one-fourth from one end of the rod and at the middle of V. These constrictions in the small autosomes seem to indicate the points of spindle-fiber attachment.

In the hybrid between the two races which has normal viability, the oogonal complex contains one heteromorphous pair composed of a V-shaped chromosome and a rod-shaped chromosome (Fig. 1, c).

### b. Salivary gland chromosomes

In both races, there are six strands branching off from the chromocenter, of which five are long and one very short. One of the long strands represents the X-chromosome.

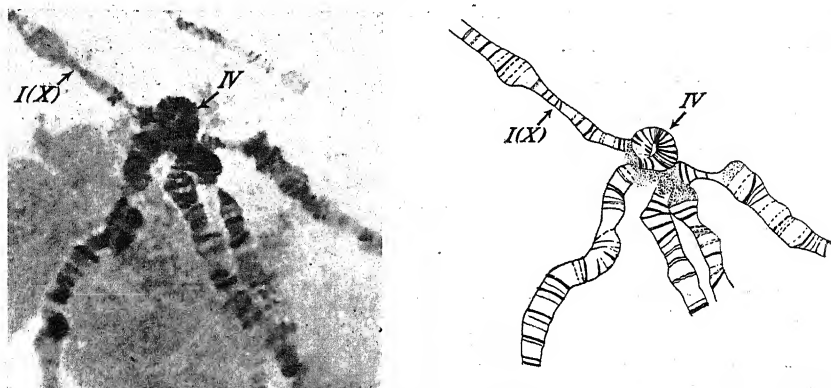


Fig. 2. Salivary gland chromosomes.

This strand exists in a slender haploid condition in the salivary gland nuclei of the male larva. The other four strands represent the arms of the long V-shaped chromosomes. The short strand is attached to the chromocenter with both proximal and distal ends and appears like a ring (Kikkawa 1936) (Fig. 2).

These observations apparently show that the small ring-like chromosome corresponds with the heteromorphic chromosome found in the meiosis, and its distal part, as well as its proximal end, is formed by inert substance.

No chromosomal aberration such as inversion and translocation is observed.

Among the strains of the species kept in this laboratory, those from Saipan (South-Sea Ids.), Matuyama (Sikoku), Yuasa, Simoda and Kabano (Honsyū) belong to B race, and the strains from Oita, Amakusa (Kyūsyū), Isigakizima (Ryūkyū Group) and Ninpo (China) belong to A race.

#### 4. Genetical Findings

So far thirty mutant characters have been obtained. Many of these were found among the progeny  $F_1$ ,  $F_2$ ,  $F_3$  or  $F_4$  of the flies treated with X-rays. Twenty-five of these have been assigned to three linkage groups. From the cytological observations stated above, four linkage groups are expected, so that there must be one group none of whose mutant genes has been discovered as yet. Presumably this missing group corresponds with the small heteromorphic chromosome indicated above.

##### a. Linkage data and chromosome maps

The map distance was determined by the recombination value between the two genes and the order of genes by means of the customary three point experiment. In some cases where no three point experiment had been performed, the order was determined by consulting various data concerning the two point experiments. Of the chromosome maps prepared in this manner those of the first and third linkage groups are relatively more complete than that of the second linkage group where it has been difficult to ascertain the loci of the mutant genes on account of the similarity of the phenotypes of many of them (Fig. 3).

##### b. Description of the mutant characters

###### Chromosome I (X-chromosome)

*rl*—rolled. (1-0.0). 39b28 Spontaneous in *vermilion*. Wing edges rolled downwards. RK1.

*Pw*—*Pointed wing*. (1-6.4) 39b15 Wings short and narrow. Wings of heterozygous female narrowed slightly at tip. Dominant hom. fully viable. RK2(♂).

*w*—*white*. (1-17.4) 38j18 Induced by X-rays. Eye color white and ocelli colorless. Rk1.

*w<sup>a</sup>*—*apricot*. (1-17.4) 39f20 Allel. of white. Eye color yellowish pink. *w<sup>a</sup>*. v. more yellowish. RK1.

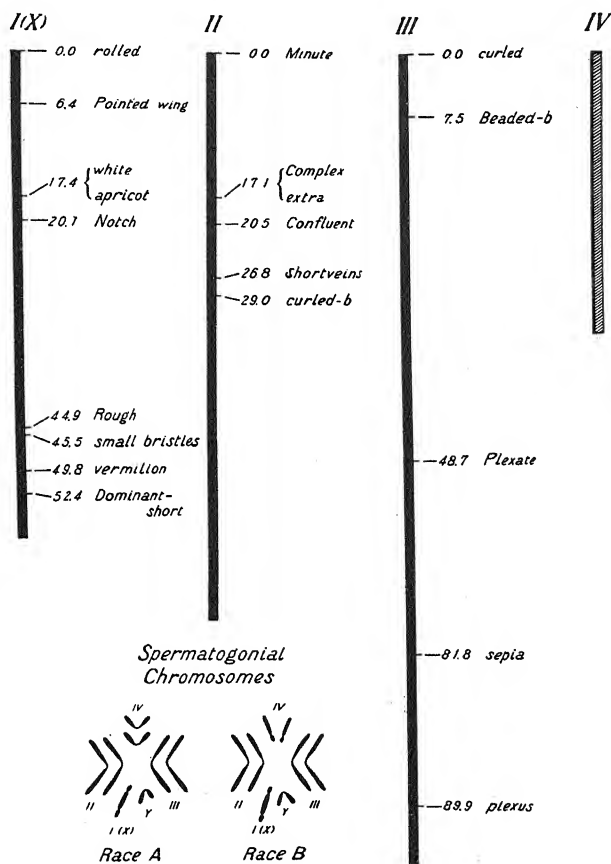


Fig. 3. The chromosome maps.

*N*—*Notch*. (1-20.1) 38e2 Ind. by X-rays. Wings notched at tips and often along edges. *L2* and *L5* thickened. Hom. ♀ and ♂ lethal. RK2.

*Ro*—*Rough*. (1-44.9) 38f11 Spon. in *Notch*. Eye surface rough with facets irregular in size and in arrangement. Eyes of hom. slightly smaller than wild-type. RK2.

*sb*—*small bristles*. (1-45.5) 38d13 Spon. in wild stock. Bristle length 1/3 of wild-type. Late hatching. RK3.

*v—vermilion.* (1-49.8) 38j29 Spon. in *Notch*. Eye color bright scarlet, not translucent; ocelli colorless. RK1.

*Ds—Dominant short.* (1-52.4) 39f5 Spon. in wild stock. *L5* shortened. The inner margin of the wings often excised. Dominant hom. ♀ and ♂ fertile. RK1.

## Chromosome II

*M2a—Minute 2a.* (2-0.0) 38f6 Ind. by X-rays. Minute bristles. Hom. lethal. Late hatching. RK3.

*Co—Complex.* (2-17.1) 38i28 Ind. by X-rays. Post-cross vein thickened like in *Plexate*. Hom. lethal. RK3.

*ex—extra.* (2-17.1) 38b5 Spon. in wild stock. Allel. of *Complex*. Post-cross vein with an extra vein, occasionally overlaps wild. RK3.

*Cf—Confluent.* (2-20.5) 38h9 Ind. by X-rays. *L2* and *L5* thickened and knotted. Wings slightly narrowed, often with a vesicle. Hom. lethal. RK2.

*Cf<sup>2</sup>—Confluent.<sup>2</sup>* (2-20.5) 39d1 Ind. by X-rays. Allel. cf *Cf*; like *Cf*. RK2.

*Sv—Short veins.* (2-26.8) 38c20 Spon. in wild stock. Veins shortened like in *Hairless*. Hom. lethal. RK1.

*cu-b—curled-b.* (2-29.0) 39c9 Spon. in *Short veins*. Wings curved upwards, occasionally throughout their length and slightly divergent. Postscutellars often erect and crossed. RK2.

*H—Hairless.* (2-?) 37i26 Spon. in wild stock. Many scutellars and dorsocentrals gone. Vein shortened, especially *L3* and *L5*. Hom. lethal. RK1.

## Chromosome III

*cu—curled.* (3-0.0) 38e6 Spon. in wild stock. Wings curved upwards like *curled-b*, but postscutellars normal. RK2.

*Bd-b—Beaded-b.* (3-7.5) 39d16 Ind. by X-rays. Wings with excisions on anterior margin, intensified by *Notch*. Hom. lethal. RK1.

*Pt—Plexate.* (3-48.7) 38i28 Ind. by X-rays. Posterior cross-vein thickened. Hom. lethal. RK1.

*se—sepia.* (3-81.8) 39e16 Spon. in curled. Eye color light brownish on emergence, darkening to sepia. Viability poor. RK4.

*px—plexus.* (3-89.9) 38h9 Ind. by X-rays. Extra veins in the neighbourhood of post-cross vein. RK2.

*ba—balloon.* (3-?) 38c8 Ind. by X-rays. Wings inflated on the inner side, narrower than in wild-type and often divergent. RK3.

*wo—white ocelli.* (3-?) 38b19 Spon. in wild stock. Ocelli colorless. Eye color normal. RK4.



**Chromosome unknown**

*Bd*—*Beaded* 38k14 Spon. in *Notch*. Wings with excisions on anterior margin. Extremely variable and often overlaps wild. Hom. lethal. RK4.

*br*—*broad*. 38? Spon. in *curved*. Wings broadened and rounded at tip. RK4.

*c*—*curved*. 38d13 Spon. in wild stock. Wings curved downwards, more pronounced in ♀. RK4.

*i*—*interrupted*. 39c1 Ind. by X-rays. P.c. absent or reduced, more pronounced in ♀. RK4.

*wp*—*warped*. 38d22 Spon. in wild stock. Wings divergent, small narrow and dusky, curved or warped. RK4.

Table 1. Summary of linkage data

## The first chromosome

Loci tested	Crossovers	Total flies	Recombination percentage
rl — w	34	195	17.4
w — N	54	2560	2.1
w — sb	218	829	26.2
N — sb	162	658	24.6
w — Ro	40	213	18.7
Ro — sb	3	650	0.4
Ro — v	30	377	7.9
v — Ds	27	1366	1.9
Ro — Pw	536	1712	31.3
w — Pw	188	1712	10.9
wa — Ro	210	1153	18.2

## The second chromosome

Loci tested	Crossovers	Total flies	Recombination percentage
Cf — M2a	6	547	1.0
Cf — cu-b	6	190	3.1
Cf — Sv	59	891	6.6
Cf <sup>2</sup> — cu-b	17	265	6.4
Co — cu-b	65	602	10.7
Sv — ex	47	484	9.7
M2a — ex	92	444	17.1
Sv — cu-b	23	1019	2.2
Cf <sup>2</sup> — M2a	36	231	15.5
Sv — M2a	133	566	23.4

## The third chromosome

Loci tested	Crossovers	Total flies	Recombination percentage
Pt — cu	266	678	39.2
Bd-b — px	168	342	49.1
Bd-b — cu	163	2203	7.3
Bd-b — Pt	920	2203	41.7
Bd-b — se	48	102	47.0
Pt — se	119	359	33.1

### 5. Non-disjunction

Females of Race B homozygous for *white* or *vermilion* (both sex-linked genes) were treated with X-rays (50KV. 4 ma. 20 cm. nonfilter 30 minutes), and mated with wild-type males. As shown in Table 2, the percentages of the exceptional females and males produced in  $F_1$  are not significantly different between treated and control strains.

Table 2. (Primary non-disjunction)

Mating		Regular offspring		Non-disjunctions		Total
X-ray treated		♀	♂	♀	♂	
	$\frac{w}{w} \times +$	606	520	0	5	1131
	$\frac{v}{v} \times +$	922	824	2	7	1746
Total		1528	1344	2	12	2886
%				0.13	0.89	
Control		2104	1932	3	19	4058
%				0.14	0.98	

Table 3. (Secondary non-disjunction)

Mating		Regular offspring		Non-disjunctions		Total
XXY $\times$ + (vermilion)		♀	♂	♀	♂	
		638	587	1	4	1216

Among the 1216 flies produced by the XXY mother, five were exceptionals, the frequency being 0.75% in the male and 0.16% in the female, and the female to male ratio 1:4. Among the daughters of such a mother, the same number of XX's and XXY's are expected. Cytological examinations showed that, of 27 tested, 10 were XX's and 17 were XXY's.

### 6. Summary

1) *Drosophila montium* is one of the tropical species distributed widely in the Eastern Hemisphere: Africa, Formosa, Java, India, Samoa, Sumatra and southern regions of Japan and China.

2) Two races, A and B, may be distinguished in this species by the difference in chromosome complex. The oogonial metaphase plate includes four pairs of chromosomes, namely, two large V-

shaped pairs, a rod-shaped pair (X's) and a pair of small chromosomes, which are V-shaped in Race A and rod-shaped in Race B. In the male, one of the rod-shaped X's is replaced by a small V-shaped Y. In the salivary gland nucleus, six strands radiate from the chromocenter. One of these is much shorter than others and is attached to the chromocenter with both ends like a ring. This apparently corresponds with the short rod and small V found in the metaphase complex.

3) Thirty mutants have been discovered, of which twenty-five are assigned to three linkage groups. The mutants are described briefly.

4) Percentages of spontaneous non-disjunctions and of those obtained by X-ray treatment are given.

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## Effects of Fast Neutrons Upon Plants, III. Cytological Observations on the Abnormal Forms of *Fagopyrum* and *Cannabis*

By

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(With 16 figures in the text)

Received February 24, 1940

In the first paper of this series a description was given of the germination rate, growth of roots and morphological changes in both seedlings and mature plants of two species, *Fagopyrum esculentum* and *Cannabis sativa*, grown from neutron-bombarded seeds (Nishina and Sinotô 1939). A preliminary description was also given of the cytological observations made on the abnormal forms of *Fagopyrum* (l. cit. pp. 726-727). In the present paper the detailed results of the cytological investigations made on the abnormal forms of the two species produced by the bombardment will be described.

### Materials and methods

All materials used in the present work were taken from the plants described in the first paper mentioned above, to which reference may be made for further details on the external morphological characters of the abnormal forms. The methods of bombardment and cytological observations have been given separately in each case.

### Description

#### *Fagopyrum esculentum* (figs. 1-10)

Dry seeds were exposed for 130 minutes to neutron rays produced by bombarding a beryllium target with 2.8 MeV neutrons from the cyclotron in this laboratory (intensity, 278 arbitrary units).

The percentages of germination were 74% in the control and 54% in the treated class and the growth of the roots was found to be better in the control than in the class exposed to the rays.

The abnormal forms which appeared are classified into seven characteristic categories as follows: 1) Many plants with variegated leaves (0.7% in the control and 74% in the treated class). 2) Plants with irregular leaves, that is notched, cleft, or deleted ones (more than 86%). 3) Plants with twisted or fasciated stems (0.28% in

the control and 23.60% in the treated class). 4) Dwarf plants with many small leaves. 5) Plants having a similar appearance to that induced by the colchicine treatment, that is, with partially or totally thickened leaves and abnormal or apetalous flowers in chimera branches. 6) Plants with only one or two thickened leaves besides two cotyledons, which can be compared with the dwarf mixoploids that appeared in the case of the colchicine treatment. 7) Plants with irregular or blue-greenish flowers, the latter being very thick as compared with normal flowers and sepalous in appearance.

Most of these types of abnormality were observed cytologically by using the acetocarmine smear method.

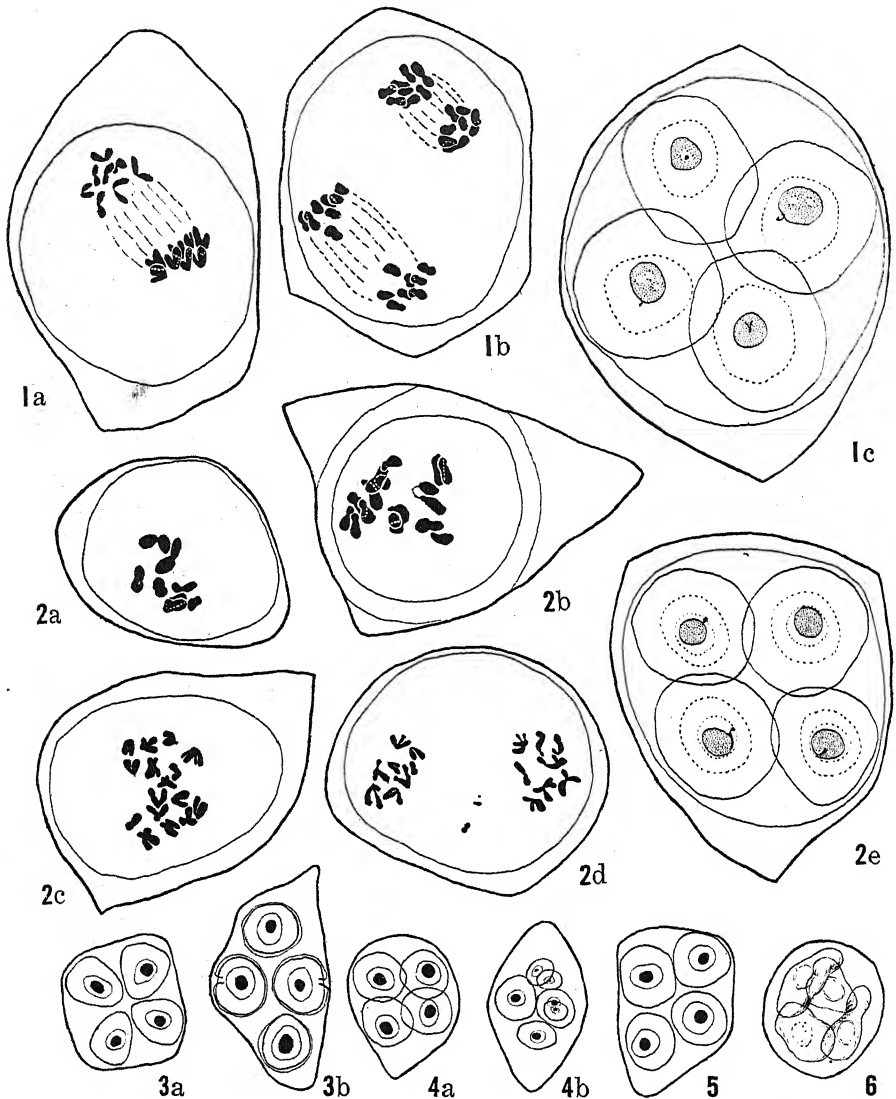
*F. esculentum* has 16 somatic chromosomes in the root-tip cells and generally shows one or two nucleoli in every nucleus. So it is easy to see that there is one pair of SAT-chromosomes or nucleolar chromosomes. The first and second meiotic divisions of the pmc's go on normally to form 8 bivalents, and 4 daughter nuclei thus formed result in quite normal quartets by the cell membrane formation (fig. 3). The nucleolus at the prophase has two deeply stained bodies or trabants and becomes attached to one pair of chromosomes. In normal microspores only one nucleolus is observed in every nucleus of quartets or later stages.

Many plants with variegated leaves were found to show no noteworthy cytological irregularities. Thus it suggests no relation to the chromosomal mutation at least, but may be ascribed either to the effects of the neutrons on the plastids or to gene mutation.

In plants with twisted or fasciated stems the first and second divisions proceeded normally, resulting in four normal quartets (fig. 1). Consequently such a morphological abnormality as fasciation is to be attributed to the physiological effect (or gene mutation) and not to the chromosome aberration. In the extreme case of this deformity however the pollen grains of such branches have a wide range of size variation suggesting cytological irregularities.

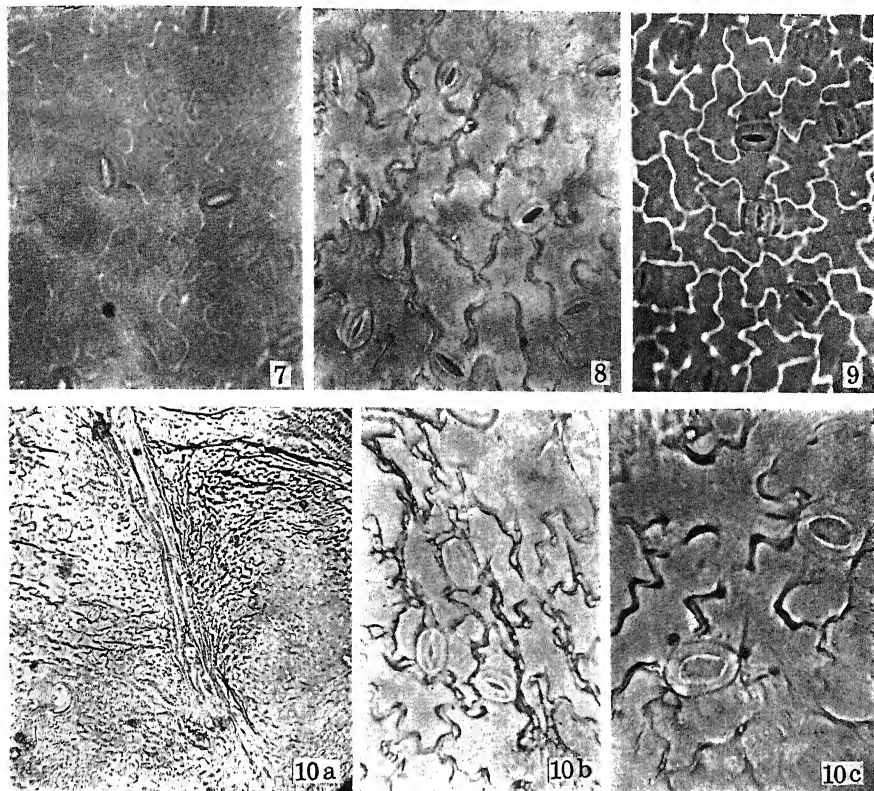
In dwarf plants with many small leaves meiotic behaviour was somewhat irregular, and fragments and bridges of the chromosomes were found though rarely (fig. 2), while polyads and many normal tetrads were also found. Accordingly, dwarf pollen grains are usually formed. It is remarkable that the sizes of the pmc's, quartets and pollen grains are smaller than those of the corresponding normal bodies (compare figs. 2 and 4 with figs. 1 and 3). This abnormality seems to be due to the chromosomal and genic mutation. The dwarf type behaves as a monogenic recessive to the usual type, according to Eghis (1925), but whether the present case is a

dominant mutation or not remains to be shown by further genetic analysis.



Figs. 1-6. The meiotic behaviour of the pmc's in buck wheat ( $n = 8$ ) developed from the dry seeds treated with neutron rays. 1, in a plant with a fasciated stem. a, first anaphase. b, second anaphase. c, normally formed quartets. 2, dwarf plant with many small leaves. Notice the differences in size of pmc's and quartets. a, first metaphase. b, polar view of early anaphase showing a chromosome bridge. c, late anaphase. d, second metaphase showing two fragments. e, normally formed quartets. 3-6, comparison of various tetrads. 3, normal control plant. 4, dwarf plant. a, normally formed quartets. b, abnormal polyads. 5, plant with partially thickened leaves. 6, plant with blue-greenish sepalous flowers indicating shrivelling quartets. 1-2,  $\times 1020$ ; 3-6,  $\times 464$ .

In plants with partially or totally thickened leaves or occasionally with abnormal or apetalous flowers, the normal or somewhat abnormal branches showed only normal tetrads (fig. 5), but definitely abnormal branches, especially those having apetalous flowers, showed large and small pollen grains similar to those which appeared in the tetraploids, octoploids or mixoploids induced by the colchicine treatment (cf. Sinotô and Satô 1939).



Figs. 7-10. Comparison of the stomata and epidermal cells of under epidermis of the leaves in various types. 7, control plant. 8, dwarf plant with many small leaves. 9, plant with blue-greenish sepalous flowers. 10, plant with partially thickened leaves. a, chimera tissue. b, diploid tissue. c, perhaps octoploid tissue. 7-9, 10b-c,  $\times 280$ ; 10a  $\times 70$ .

In plants with only two or three leaves, abnormal or apetalous flowers were formed or sometimes not even such abnormal flowers developed, this being perhaps due to the unbalanced relations existing between chromosomes and cytosome. The dwarf mixoploid induced by the colchicine treatment were also explained in the same way (Sinotô and Satô 1939).

In plants with blue-greenish and sepalous flowers, the abnormality was not so remarkable; nevertheless shrivelling quartets were often found (fig. 6). This cytological irregularity may be attributed not to a chromosome aberration, but to cytoplasmic influence. Whether this type is a dominant gene mutation or not, cannot be determined until later generations are analyzed genetically.

The stomata in the under epidermis of the leaves were printed on a film on which a drop of amyl acetate was placed beforehand (a simple modification of the SUMP method) and then these prints were examined under the microscope. In this case the second or third young leaves from the top of the plants were selected for observation in order to avoid variation of the stoma size.

Since differences in the stomata of the under epidermis of the leaves taken from these abnormal plants cannot be so clearly distinguished except in the case of chimera plants (figs. 7-10). Dwarf plants have smaller pmc's and pollen grains than the control plants, but there is no apparent difference in the stoma size, although the membranes of the epidermal cells are thicker and more irregular than those of the control ones (figs. 7, 8). Such irregular epidermal cells are clearly observed in other abnormal types. In the case of the plants with partially thickened leaves the differences in stoma and cell sizes are clearly demonstrated in fig. 10. From the results of the observations made in polyploid or mixoploid plants induced by the colchicine method, it may be inferred in the present case that the large stomata are tetra- or octoploid ones, while the small stomata are normal diploid ones (cf. Sinotô and Satô, 1939). Such chimera plants may be derived from the polyploid and diploid tissues affected by the neutron rays, after recovery of the balance between the chromosomes and cytosome. When the balance is not restored the plant may not be developed further than the stage of two or three leaves, this being one of the abnormal types.

#### *Cannabis sativa* (figs. 11-16)

The seeds after being soaked for 12 hours in water were irradiated by the neutron rays for 120 minutes (intensity, 260 arbitrary units).

The germination rates were 89.2% in the control and 87.3%, 87.2% and 85.4% respectively in three sets of the treated class (I. II. III.)<sup>1)</sup> The difference in growth between the control and treated classes was not so clearly distinguished. A number of

1) The average distances from the beryllium target of these sets of seeds were ca. 10 cm (I), ca. 7 cm (II) and ca. 3cm (III) respectively.



abnormal forms appeared, especially in the case of a strong dose (III). These abnormal types are divided into the following five categories: 1) Plants with variegated leaves (in the case of the first leaves, 0% in the control and 100% in all three treated classes). 2) Plants with deleted or deformed leaves. 3) Plants with fasciated stems. 4) Dwarf plants. 5) Dwarf plants with fasciated stems (0% in the control, 1.2% in I, 0.7% in II and 21.2% in III). 6) Plants which had only the first leaves and had withered at an early developmental stage (0% in the control, 1.7% in I, 2.4% in II and 15.7% in III).

All of the seedlings grown from the treated seeds showed spot-variegation in all the leaves, this being especially marked in the case of the strong dose (III). Such types of variegations and wrinkling of the leaves did not appear in the leaves at a later stages of development of the plants, but instead other types of variegation, mainly sectorial in nature, appeared. The size of the variegated area and the actual colours were found to vary.

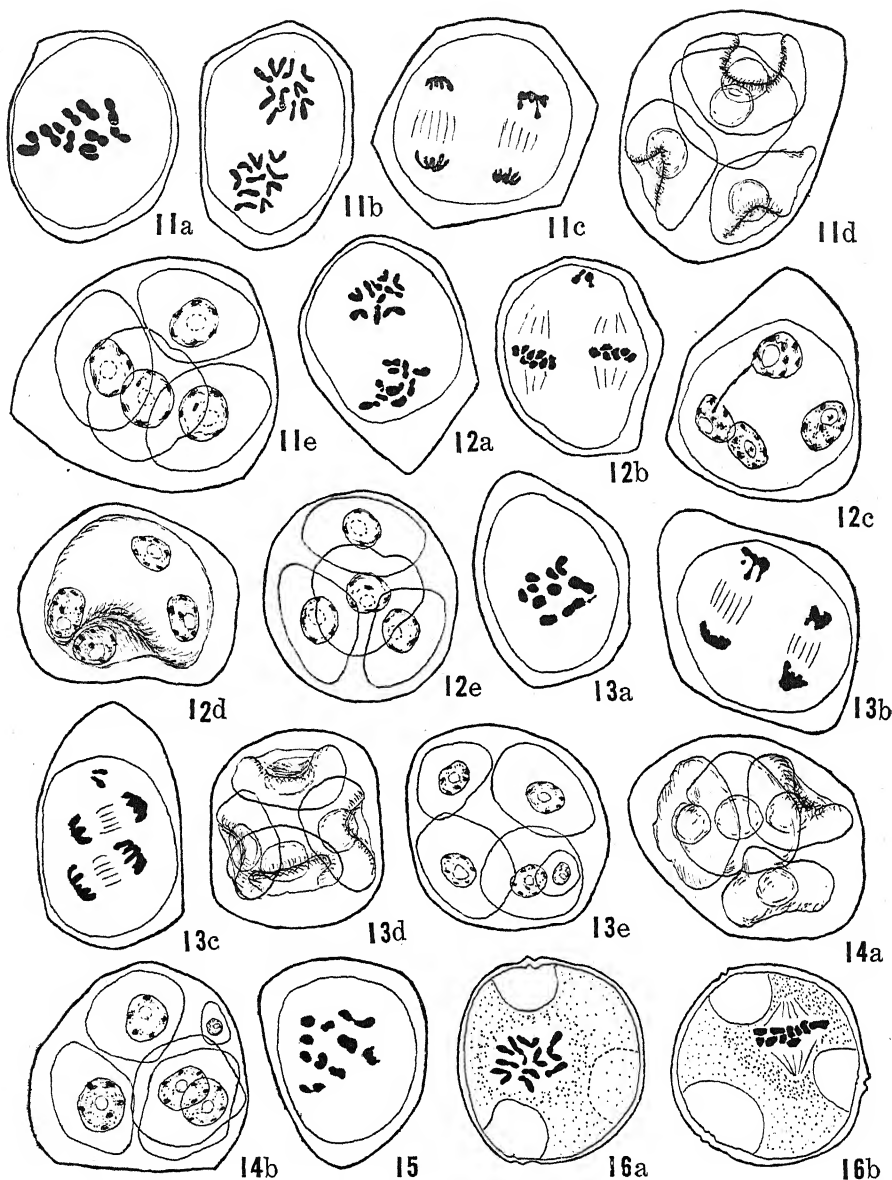
In the first meiotic division of the variegated forms 10 bivalents are regularly formed and the second division is also carried on normally, resulting in four normal quartets, but some of these are abnormal or shrivelled, which may be attributed to the influence of the neutrons on the cytoplasm (fig. 11, d, e).

In plants with fasciated stems, the abnormal chromosome behaviour is rarely observed. The chromosomes (probably not fragments) wandered into the cytosome and very rarely formed a bridge (fig. 12, c). Such abnormal behaviour of the chromosomes may depend upon the abnormal spindle mechanism or karyoplasmic disturbance due to the effect of the neutrons; consequently abnormal, especially shrivelling quartets were often found.

In the dwarf plants the irregular meiotic behaviour is more frequently observed than in the plants with fasciated stems and shrivelling tetrads and polyads were found in addition to normal ones (fig. 13, d, e).

In dwarf plants with fasciated stems, chromosome irregularities seem to have occurred more frequently than in the case of the dwarf plants with normal stems. Shrivelling tetrads and polyads were frequently observed (fig. 14, a, b).

In those plants which had only the first leaves and withered in an early stage of development it is probable that the physiological balance between chromosomes and cytosome was disturbed by the neutrons which rendered further development impossible. In the case of hemp seeds treated with colchicine, the affected seedlings with thickened leaves and stem (perhaps polyploid or mixoploid)



Figs. 11-16. Meiotic behaviour of the hemp ( $n=10$ ) raised from the wet seeds (12 hours in water) irradiated by neutron rays. 11, plant with variegated leaves. a, first metaphase showing 10 bivalents including one allosome pair. b, second metaphase. c, second telophase. d, shrivelling quartets. e, normal quartets. 12, plant with fasciated stem. a, second metaphase. b, abnormal second metaphase showing two chromosomes in the cytosome. d, shrivelling quartets. e, normally formed quartets. 13, dwarf plant. a, first metaphase showing fragment. b, second telophase. c, abnormal second telophase showing two chromosomes thrown out in the cytosome. d, shrivelling quartets. e, pentad. 14, dwarf plant with fasciated stem. a, shrivelling quartets. b, hexad. 15, first metaphase especially showing a heteromorphic bivalent. 16, first metaphases in pollen grains of the control plant. a, polar view. b, side view.  $\times 1020$ .

frequently withered at the development stage when they had their first leaves. A similar fact was also observed in the case of root-tip cells of *Vicia faba*. Its meristem is the most sensitive to the irradiation of the neutron rays by which cell divisions were very much disturbed in spite of the activation of the cell elongation process. Consequently in the case of strong exposure the seedlings of *Vicia* could not continue further development and withered soon after (cf. Nishina, Sinotô and Satô, 1940).

The first division of the pollen grains was observed, in order to detect the chromosome aberration, which however proved difficult to make out. The pollen grain has three protuberances in the same plane, namely in the plane of the slide glass. Accordingly, most metaphase plates were found to offer a side view which rendered the observation of chromosome aberrations difficult even if they were brought about by the action of neutrons. Fig. 16, a, b show 10 chromosomes in the pollen grains of the control plants with an indication in *a* of the characteristic constrictions of their spindle fiber attachment.

### Conclusion

The irradiation of seeds with neutron rays has induced various chromosome aberrations, namely chromosome doubling, translocation, delation and fragmentation, most of which seem to have been restored or eliminated in a few cell generations. In hemp which grew over 3 meters in height, such chromosome aberrations could not be detected in the pmc's, while in buck wheat (0.6–0.7 meters in height) such tissues developed and formed abnormal or apetalous flowers.

This work was carried out according to the program of the Atomic Nucleus Sub-Committee of the Japan Society for the Promotion of Scientific Research to which we wish to express our gratitude. We are indebted to the Japan Wireless Telegraph Company for the electromagnet and other pieces of equipment used for the cyclotron, and to the Mitsui Hōonkwai Foundation, the Tokyo Electric Light Company, and Mr. G. Hattori, President of K. Hattori and Company, for financial aid. We acknowledge the kind assistance given by our colleges of the Nuclear Research Laboratory in connexion with the irradiation of samples.

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# Morphological and Cytological Studies in the Genus *Calceolaria*

## Part II. Meiosis in diploid and aneuploid *Calceolarias*<sup>1)</sup>

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### Contents

	Page
1) Introduction . . . . .	467
2) Technique . . . . .	468
3) Meiosis in the diploids . . . . .	468
4) Discussion . . . . .	478
a) Secondary association . . . . .	478
<i>C. mexicana</i> Benth. . . . .	481
5) Introduction . . . . .	481
6) Materials and Methods . . . . .	482
7) Description of meiosis . . . . .	482
8) Discussion . . . . .	485
9) Summary . . . . .	487
10) Literature cited . . . . .	488

**Introduction.** *Calceolarias* are favourite garden plants and are mainly grown in greenhouses, except for the bedding varieties. Owing to the facility with which they hybridise, an endless number of varieties have been raised by leading horticulturists in Great Britain. A number of these varieties were raised at the John Innes Horticultural Institution. I am indebted to the late Dr. E. J. Collins of that Institution, who placed at my disposal an interesting series of varieties. Nearly all of these have been examined for their somatic chromosomes, which form the subject of another paper. Here an account is given of the details of meiosis in the diploid *Calceolarias*. *Calceolarias* have been garden favourites for years now and it is difficult to know how pure even the supposedly pure species are. However, I am told that one of the diploids is a pure species. Meiosis in the genus *Calceolaria* has not been studied before. Besides the John Innes Horticultural Institution, I have collected material from the Botanic Garden, Cambridge. To the Directors of these two institutions my grateful thanks are due.

Most *Calceolarias* are propagated vegetatively by stocks. The stocks are raised early in December. The plants start flowering in early spring and remain in bloom for about two months.

1) Part II of thesis approved for the degree of Doctor of Philosophy of the University of London.

**Technique.** Before the flower buds were fixed the floral envelopes were clipped off, in order to secure proper fixation. The two anthers were exposed and then the whole bud dipped in Carnoy (Semmens, 1937) for 25 seconds, rinsed in water and then passed on to the fixative. Belling's formula for Navashin was used throughout and gave uniformly good fixation. Where osmic fixatives were employed (Medium Flemming and Benda), the slides were bleached in three parts of 80% alcohol and one part of  $H_2O_2$ . The process of bleaching is considerably hastened by adding a pinch of lithium carbonate. If the jar is left on a warm plate, the sections are bleached in about 80 minutes. I found it worth while to use the  $H_2O_2$  in lower grades of alcohol, which saves a lot of time and incidentally eliminates the risk of the sections being washed off. When fixed in Navashin the cytoplasm invariably acquired a yellow hue, which is undoubtedly due to inadequate washing. About 6 – 8 hours gave good fixation, but the buds must be thoroughly washed in water to take off the chromic acid stain. This could be done efficiently by using tepid water. After this the buds can be transferred straight to 40% alcohol and brought to 70% alcohol the same day. Xylol proved to be a better clearing agent than chloroform. The buds were sectioned at  $14\mu$  and stained according to Newton's iodine-gentian-violet schedule. Some sections were stained with decolorised fuchsin. Hydrolysis for 20 minutes in normal HCl proved to be adequate. Slides which were taken through grades of acetic alcohol after bleaching in a solution of metabisulphate were definitely improved in the tone of the stain. It is necessary to use a fresh solution of fuchsin, as otherwise the stain diffuses into the cytoplasm. For the study of meiosis in plants with short chromosomes the Feulgen technique seems to hold no special advantage. When studying side views of metaphase with chromosomes overlapping, preparations with gentian violet are superior, on account of the transparency which they impart to the chromosomes.

**Meiosis in the diploids.** The course of meiosis in the three diploids is essentially the same, gross irregularities being occasional. A detailed description will be given for one of them and the others will be referred to where deviations from the type were observed.

Stages earlier than diakinesis were fixed well, but critical observation was not attempted owing to the small size of the chromosomes. But from what observation was made at this stage, the pairing of the double threads at zygotene might begin anywhere along the length of the threads. The observations made in this Laboratory from time to time have left no doubt that the chromosome

is essentially double throughout the nuclear cycle. (Koshy, 1934; Mensinkai, 1938, and others).

A number of cells at pachytene had one bivalent attached to the rather large nucleolus. (Figs. 1 and 2). This would correspond to the pair of satellited chromosomes in the somatic complement. The nucleolus has a prominent vacuole. At pachytene the threads are distinctly thicker, but still too long to enable the identification of the separate bivalents. Pairing seems to be quite complete, even in the region of the nucleolus. The satellites are small and borne on long threads, the two satellites being slightly divergent.

During pachytene the chromatids exchange partners at several loci, these representing chiasmata. Figs. 4, 5 and 6 show different nuclei at mid-diplotene. These configurations clearly illustrate normal chiasma formation. The variations also show that the formation of chiasmata along the paired pachytene threads is random (Cf. Campanula, Gairdner and Darlington, 1931). One bivalent is attached to the nucleolus at diplotene. (Figs. 4 and 6). In both these cases the region of attachment may include the end of the chromosome, together with the satellite, which is not visible. The maximum number of chiasmata at late diplotene is four. At this stage a number of bivalents show the eight-type of configuration with one interstitial and two terminal chiasmata. (Figs. 6 and 8). Sax (1930) attributes the loss of some interstitial chiasmata to a rupture of the crossed chromatids. Frequently at this stage the bivalents have only one interstitial chiasma. Pairs united by one chiasma may have the node at any point along the chromatids; or the association may be strictly terminal, in which case the ends of the two paired chromosomes are frequently not in contact with one another. When there is a subterminal interstitial chiasma, the sister chromatids are sometimes separated from each other for some distance along each chromosome, as shown by Sax (1930) in *Secale*. The repulsion at diakinesis is very obvious from fig. 32, and the bivalents are arranged at the periphery of the nucleus. They appear as V's, X's, O's or rods. The bivalent attached to the nucleolus at this stage is shown in figs. 23 and 24. At diakinesis an analysis of fifty bivalents taken at random showed the following distribution of the different types of bivalents:—

It is obvious that interstitial chiasmata persist in about 35% of the cases.

While most of the cells had nine bivalents, occasionally trivalents and quadrivalents were noticed.

Table 1

X	V	O	Y	Total
17	23	8	2	50
34	46	16	4	%



All drawings (Figs. 1-126) were made at desk level with an Abbe Camera lucida. An achromatic objective N.A. 1.3 was used in conjunction with a Zeiss ocular K. 30, giving a total approximate magnification of 2700 diameters, except figs. 108, 117, 118, 119, which are  $\times 1800$ . The drawings reduced to a half in reproduction.

Figs. 1-25. *C. clibrani*. 1, 2. Early pachytene showing one bivalent attached to the nucleolus. The satellites are slightly divergent. 3. Early diplotene showing nine bivalents. 4, 5, 6. Mid-diplotene nuclei drawn separately. In figs. 4 and 6 the bivalent attached to the nucleolus is indicated. The bivalents are of various shapes, with an interstitial chiasma in many of them. 7. Mid-diplotene with nine bivalents *in situ*. 8. Mid-diplotene drawn separately. One of the bivalents showing the 8-type of configuration. Another has four chiasmata, the maximum number observed at this stage. 10. Late diplotene. 11. Early diakinesis with one trivalent of the frying-pan type and one univalent. 12. Early diakinesis. 13. Early diakinesis with one trivalent and one univalent. Two bivalents which were interlocked earlier are now seen separating. 14. Early diakinesis with one trivalent and one univalent. 15.



In fifty cells at diakinesis only seven trivalents and six quadrivalents were seen. The trivalents were of the ring and rod type ("frying pan") (Figs. 11, 13, 14, 17, 19, and 22) involving one simple terminal chiasma and one triple chiasma. The other observed form of trivalent is the ring trivalent. (Figs. 19 and 26). This type of configuration can arise if we assume segmental interchange (Belling, 1927) resulting in one of the chromosomes of the ring having two homologous ends.

Quadrivalents are less frequent and the types occurring are illustrated in figs. 20, 25, 27 and 28. The association of more than two chromosomes in a diploid has been described in several genera: *Tradescantia*, *Rhoeo*, *Datura*, *Pisum*, *Campanula*, *Zea*, *Matthiola*, *Oryza*, *Anthoxanthum*, *Rosa*, etc. If the side-by-side conjugation of homologous chromosomes is a universal condition of meiosis, then rings of four or more chromosomes in a diploid must be due to segmental interchange between non-homologous chromosomes, as suggested by Belling and Blakeslee (1924, 1926) and Belling (1927) with respect to *Datura*. The occurrence of multivalents indicates that *Calceolaria elibrani* is a structural hybrid. The low frequency of multivalent formation may be due to the smallness of the exchanged segments, since chiasma formation is fortuitous and random.

In some cells at diakinesis two bivalents were found in which the pairs were not connected by chiasmata but were interlocked at diplotene. (Fig. 16). According to Catcheside (1931), interlocking is a mechanical result of the pairing of the chromosomes commencing at the ends during synapsis. Interlocked bivalents not connected synaptically have been observed in *Datura* (Blakeslee, 1929), *Hya-cinthus* (Darlington, 1929), *Oenothera* (Gates and Goodwin, 1931; Hedayetullah, 1932; Catcheside, 1931), *Scilla* (Dark, 1926). They have also been found in certain Orthoptera. This type of interlocking is usually undone by metaphase and so does not cause irregularities at Anaphase I. In fig. 13 two ring bivalents which were presumably interlocked are now seen separated.

As the nuclear membrane disappears, the repulsion which is at its peak at diakinesis weakens and the chromosomes are grouped in the centre. This stage has been called prometaphase by Lawrence

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Early diakinesis showing nine bivalents. Interstitial chiasmata are still seen persisting in two bivalents. 16. Early diakinesis showing two interlocked bivalents. 17. Early diakinesis showing one trivalent of the triple arc-type and one bivalent. 18. Early diakinesis showing nine bivalents. 19. Early diakinesis with one ring trivalent. 20, 21. Early diakinesis with one quadrivalent. 22. Late diakinesis with one trivalent. Interstitial chiasmata still persist in two bivalents. 23, 24. Late diakinesis. One bivalent is attached to the nucleolus. 25. Late diakinesis showing one quadrivalent.

(1931a). In polyploids the homologous bivalents which have failed to pair at zygotene have a chance to associate themselves if they happen to lie within the orbit of attraction of one another. This tendency becomes obvious even at late diakinesis. (Fig. 33).

Nine bivalents are easily recognised in polar views of Metaphase I. In fig. 36 one of the bivalents still shows the satellite and its thread. This condition is rare, as progressive linear contraction of the chromosome usually results in a 'retraction' of the satellite. Fig. 37 shows a polar view of Metaphase I where the nine bivalents are completely free from one another. Here two bivalents are in the centre and the other seven all round. This would be the stable form of arrangement in a plate with nine bivalents of equal size on the basis of Mayer's experiments with floating magnets. Several workers have attempted to correlate the arrangement of chromosomes on the metaphase plate with the positions occupied by floating magnets in a field. We may mention Cannon (1923), who gave several figures indicating a close resemblance between the arrangement of floating magnets and chromosomes on the metaphase plate. Kuwada (1929) and his collaborators undertook an exhaustive survey of this problem with model experiments supported by statistical investigations. Kuwada assumes that all chromosomes are electronegative in the nucleus; thus they repel each other (Gates 1909). When the nuclear membrane disappears at the end of the prophase and the chromosomes are exposed directly to the cytoplasm, the electronegative chromosomes are attracted to the electropositive equatorial region of the cell and at the same time they are repelled by the poles of the same sign of charge as their own so that they tend to form the nuclear plate. Through the repellant action exerted between the chromosomes and both poles of the spindle, the chromosome arrangement will tend to form a simple ring. So in metaphase the chromosomes repel one another and at the same time are attracted by both poles of the spindle. In the case of floating magnets also, each magnet repels the others, and at the same time is repelled by both electromagnetic poles. The attraction-repulsion relation is the same in both cases. Thus, the final distribution of chromosomes in the equatorial plate presents the same figure as those obtained with floating magnets. When all the chromosomes are distributed in the equatorial plate, the resemblance is very striking. The same is true when the chromosomes are small with no size differences.

The arrangement described in the case studied, viz. two in the centre and seven in the periphery, would be in accordance with the theoretical expectation. Catcheside (1937) found a similar arrangement in *Brassica oleracea*. But where the bivalents were associated

the arrangement differed widely from the expected arrangement, as Nandi (1936) found in Rice. Nandi states that associated bivalents behave as units, so preserving the arrangement comparable to floating magnets. Alam (1936) made a similar observation to mine, in *Brassica*.

A summary of the observed kinds of secondary pairing is appended in Table 2. The most frequent arrangement is 3 (2) + 3 (1).

Of the ten different types of association, four seem to have a higher frequency than the others. Catcheside (1937) found in *Brassica* that there were ten kinds of secondary pairing, of which four showed a higher frequency. He regards the aberrant cases as due to three causes: (1) inferior fixation, causing false associations, (2) faulty observation, and (3) structural complexity, especially re-duplication within the complement, in addition to secondary polyploidy. But he thinks that the first two causes cannot be of importance.

The maximum association observed is 3 (2) + 1 (3), indicating that four is the basic number from which the cardinal number nine has been derived.

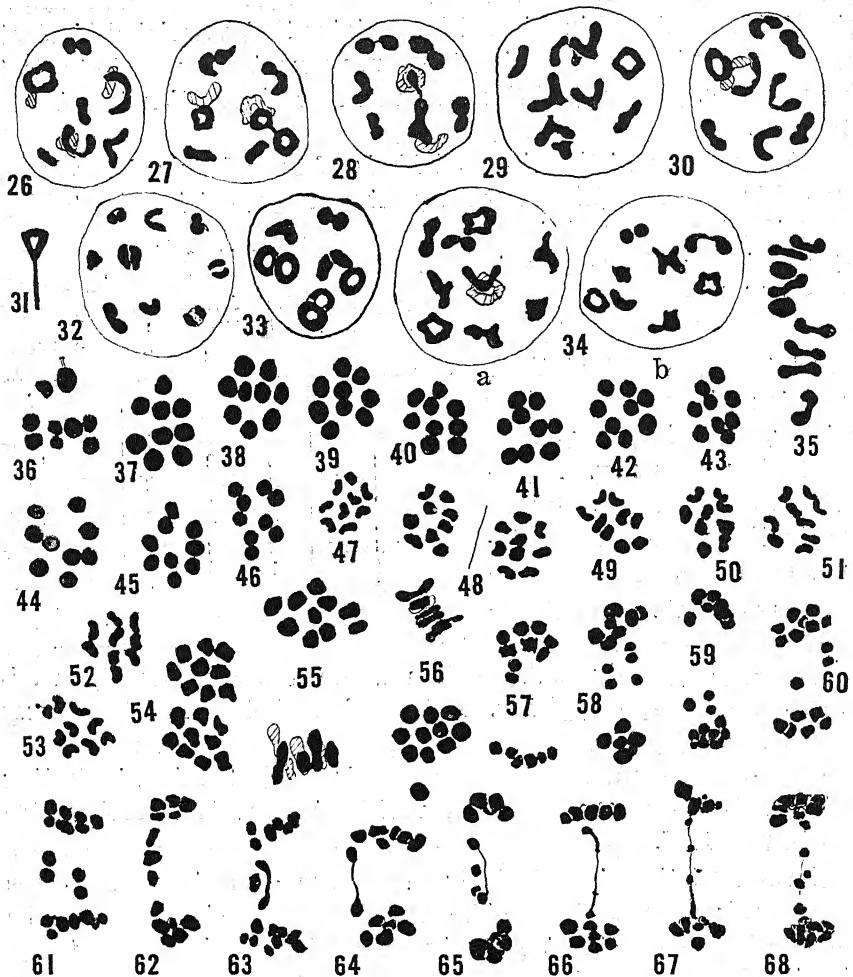
In fig. 35 the nine bivalents are drawn separately. They are usually rods with a single chiasma. Terminalisation seems to be more or less complete at metaphase; lagging bivalents due to an interstitial chiasma being rare. The chiasma frequency at metaphase is 1.38 per bivalent.

In a few plates at Metaphase I, the number of bivalents was more or less than nine. In fig. 55 the metaphase groups of two adjacent P.M.C.s are shown, having respectively ten and eight chromosomes. This condition must have arisen by non-disjunction in the last premeiotic mitosis. Similarly, fig. 56 shows two adjacent P.M.C.s with eleven and seven chromosomes.

Hedayetullah (1932) found in the hybrid *O. rubricalyx* × *O. eriensis* a mother cell with fifteen chromosomes adjacent to one with thirteen chromosomes. It has been reported in several other cases: *Oenothera* (Gates and Sheffield, 1929), *Hypericum* (Hoar, 1931), *Drosophila* (Bridges, 1913), *Datura* (Belling and Blakeslee, 1924), *Uvularia* (Belling, 1925), *Nicotiana* (Goodspeed, 1923; Ruttle, 1927).

Table 2

No. of secondary association	No. of bivalents in association			No. of cases
	1	2	3	
0	9	—	—	7
1	7	1	—	9
1	6	—	1	3
2	5	2	—	17
2	3	—	2	2
2	4	1	1	11
3	3	3	—	20
3	2	2	1	7
4	1	4	—	1
4	—	3	1	4
				Total 81



Figs. 26-68. 26. Late diakinesis with one ring trivalent. 27. Late diakinesis with one quadrivalent. 28. Late diakinesis showing nine bivalents. 29, 30. Late diakinesis with one ring bivalent in each cell. 31. Trivalent of the frying-pan type drawn separately. 32. Late diakinesis showing marked repulsion between the bivalents. 33. Prometaphase. The beginnings of secondary association between some of the bivalents can be seen. 34. Two adjacent cells at diakinesis showing pre-meiotic non-disjunction. The cell to the left has ten bivalents and the cell to the right has eight bivalents. 35. Side view of Metaphase I drawn separately. The bivalents are mostly rods. 36. Polar view of Metaphase I. One bivalent has a satellite. 37. Polar view of Metaphase I showing nine bivalents. Two in the centre and the other seven in the periphery, which is the stable arrangement. 38-45. Polar views of Metaphase I showing different degrees of secondary association. 46. Polar view of Metaphase I showing maximum association of bivalents.  $3(2)+1(3)$ . 47. Polar view of Metaphase II showing the stable arrangement. 48-51. Polar views of Metaphase II showing different degree of secondary association. 52. Polar view of Metaphase II showing maximum association.  $3(2)+1(3)$ . 53. Polar view of Metaphase II. One chromosome has a satellite. 54. Two separating Anaphase I groups to show that secondary association does not persist at this stage. 55. Metaphase

Premeiotic non-disjunction in the plant studied is not frequent, as only six cases were found out of more than five hundred cells. Sometimes this is detectable even at diakinesis, as in fig. 34.

Separation at Anaphase I is normal except for occasional lagging of bivalents due to interstitial chiasmata. (Fig. 63, 64 and 65). In fig. 54 are two separating daughter groups and it is clear that there is no sign of any association between the bivalents. Darlington (1937) found in *Tradescantia* the chromosomes arranged in the form of a ring and he thinks that this arrangement is due to the persistence of polar repulsion. The causes underlying anaphasic movement of chromosomes are controversial. Kuwada (1929) believed that anaphasic separation was due to the force of attraction from the poles. Darlington (1932b, 1937b) explains the movement of chromosomes during anaphase as due to the cumulative effect of three factors: (1) repulsion between the centromeres, (2) narrowing and axial stretching of the spindle and (3) weakening of repulsion from the poles. The idea that anaphasic separation is determined by the mutual repulsion of the centromeres and later by the stretching of the central region of the spindle was first put forward by Bělař (1929) and then extended by Darlington (1936). The differences in behaviour between short and long chromatid bridges between early and late anaphase have been put forward as supporting the idea that the centromere leads in separation, assisted later by the stretching of the spindle.

Figs. 58 and 59 show dividing univalents. The products of divisions may be included in the same daughter nucleus, thus leading to the formation of gametes with unequal chromosome numbers. In fig. 62 the two daughter groups are linked up by four bivalents stretching from pole to pole.

A few cells at Anaphase I show the presence of bivalents with inverted segments which form dicentric bridges and acentric fragments. (Figs. 66, 67 and 68). A bridge at Anaphase I can arise

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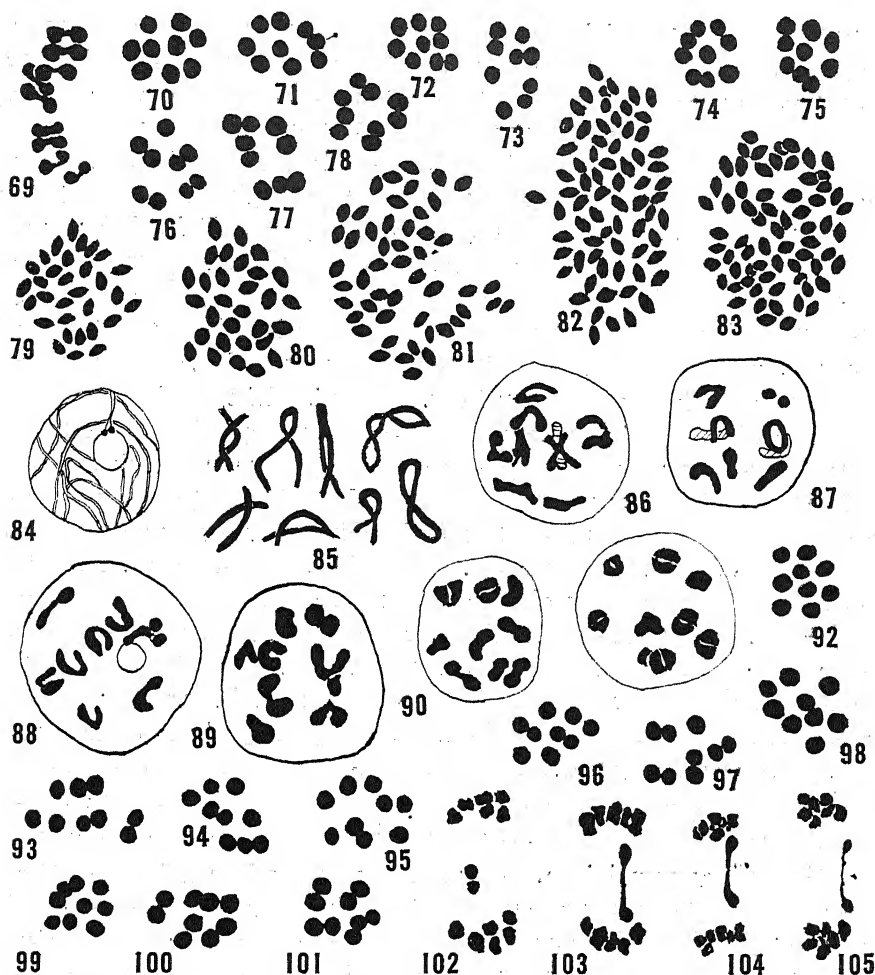
groups of two adjacent p.m.c. having respectively ten and eight chromosomes, which is the result of premeiotic non-disjunction. The lower cell is in polar view and the upper cell in side view. 56. Same as above. The lower cell has eleven chromosomes and the upper cell which is in side view has seven chromosomes. This is due to double non-disjunction. One of the chromosomes in the lower cell has been knocked out by the knife. 57. Shows a dividing univalent. The products of the division may be included in the same nucleus and give rise to unequal numbers. 58-61. Lagging univalents dividing on the spindle. 62. Two early telophase groups bridged by a string of chromosomes. 63. First anaphase with a lagging bivalent due to an interstitial chiasma and a dividing univalent. 64, 65. First anaphase, showing lagging bivalents forming a chromatid bridge due to a persisting interstitial chiasma. 66. First anaphase, showing a dicentric chromatid bridge. The fragment has disappeared. 67. First anaphase, showing a dicentric chromatic bridge and a small fragment. 68. Same as above. The fragment larger in size.

from the following causes, (1) a single chiasma in the inverted segment, (2) two disparate chiasmata and (3) a chiasma in the inversion and one proximal to it and comparate. The bridge is fairly long and connects the two poles, indicating that the inverted segment is fairly long. The size of the fragment varies, depending upon the position of the inversion with reference to the centromere. If the inversion is nearer the centromere, the fragment is large (Fig. 68) but if the inversion is away from the centromere the fragment is small. (Fig. 67). The bridge usually breaks under tension. The presence of inverted segments indicates that structural changes have taken place in the chromosomes. The general significance of the bridges is discussed elsewhere.

Telophases are normal. An interkinetic nucleus is organised with chromosomes uniformly distributed as at diakinesis. They show repulsion, as can be inferred by their disposition. The interkinesis seems to be of some duration, which results in an elongation of the chromosomes in the second division. In the second metaphase the spatial relationships of the spindles are very variable. Polar views of Metaphase II show various degrees of secondary association covering the same range of types as Metaphase I. Catcheside (1937) found in *Brassica oleracea*, a positive correlation between the two Metaphase II plates. He analysed the variation into two parts between and within the pollen mother-cells and found less variation within than between mother-cells. From this he concludes that the chromosomes at late interphase are influenced in their arrangement by that of the previous metaphase. He thinks that there is little movement of the chromosomes relative to one another at interphase. In the present case the type of association exhibited by the two plates in a cell varies very much and there is no constancy in the type of association either. In fig. 53 one of the chromosomes in Metaphase II still has its satellite.

Tetrad formation is regular and the pollen grains are binucleate at the time of discharge.

Meiosis in the other diploids follows essentially the same course. A side view of Metaphase I in *C. dentata* is given in fig. 69. All the bivalents are rods, indicating that terminalisation of chiasmata is complete at metaphase. Fig. 70-78 illustrate the different types of secondary association at Metaphase I and are similar to the ones found in *C. elibrani*. The maximum association is 3 (2) + 3 (1). (Fig. 78). Very often somatic cells of the anther in the vicinity of the loculus had the doubled and quadrupled number of chromosomes and the chromosomes were spindle-shaped. (Figs. 79-83). These have probably arisen by failure of anaphasic separation in a



Figs. 69-83. *C. dentata*. 69. Side view of Metaphase I drawn separately. All the bivalents are rods. 70. Polar view of Metaphase I showing nine bivalents with two in the centre and seven in the periphery. This is the stable arrangement. 71-77. Polar views of Metaphase I showing different degrees of secondary association. In fig. 71 one of the bivalents has a satellite. 78. Polar view of Metaphase I. The nine bivalents arranged in four groups, 3(2)+1(3), which is the maximum association. 79-83. Somatic cells in the tissue of the anther showing doubled and quadrupled number of chromosomes. Figs. 84-105. *C. Banksii*. 84. Early pachytene with one bivalent attached to the nucleolus. The satellites are prominent. 85. Mid-diplotene to show the different types of configuration. One bivalent has four chiasmata which is the maximum number at this stage. 86, 87. Early diakinesis showing nine bivalents in each cell. 88. Early diakinesis. One bivalent is attached to the nucleolus. 89, 90. Late diakinesis with nine bivalents. 91. Late diakinesis showing repulsion amongst the bivalents. 92. Polar view of Metaphase I showing the stable arrangement of the bivalents. 93-101. Polar views of Metaphase I showing different degrees of association. The maximum association of 3(2)+1(3) is in fig. 101. 102. First anaphase with a lagging bivalent splitting. 103, 104. First anaphase with lagging bivalents due to interstitial chiasmata. In fig. 104 a fragment is seen in addition. 105. First anaphase showing a dicentric bridge and an acentric fragment. The bridge is short.

previous mitotic division. When the chromosomes undergo a second split a tetraploid nucleus would form. de Litardière (1923) observed in the roots of *Spinacia* that the chromosome halves separated during the prophase and underwent a second splitting so that each telophase nucleus received the tetraploid number rather than the diploid. Other probable cases of "non-division" are *Cannabis* (de Litardière, 1924; Langlet, 1927), *Lycopersicum* (Lesley, 1925) etc. It is obvious that such cells would not be of any consequence unless they were in the lineage of reproductive cells.

Fig. 84 represents a pollen mother-cell of *C. Banksii* at pachytene. One bivalent is found attached to the nucleolus. The satellites are borne on long threads as in *C. ciliolabris*. The configurations at diplotene present the same patterns as in the type described. (Fig. 85). The maximum number of chiasmata at this stage is four. One bivalent is attached to the nucleolus at diakinesis. (Fig. 88). The bivalents at diakinesis have terminal chiasmata and show the characteristic repulsion of this stage. (Fig. 91). Secondary association at Metaphase I is very obvious, the maximum association being 3 (2) + (3). (Figs. 92-101). Lagging chromosomes at anaphase are rare. A lagging bivalent is shown in fig. 103.

A dicentric chromatid bridge is illustrated in fig. 105 with a small fragment. The bridge is short, due perhaps to the small size of the dislocated segment.

### Discussion

**Secondary association.** It is now a more or less well established fact that we have to reckon with two kinds of association during meiosis; viz. (1) primary association and (2) secondary association. Primary association is believed to be the result of exchange of chromatids at the prophase of meiosis, while secondary association results from a residual attraction between different chromosomes. Association of bivalents at Metaphase I was first observed by Kuwada (1910) in *Oryza*, but at that time its meaning was not understood. It is obvious from the works of several authors since then, but its real significance as an expression of ancestral homology of the associated bivalents was first realised by Lawrence (1931), when he co-ordinated the facts relating to this phenomenon. Secondary association as an indication of ancestral homology has led to several important results and has enabled a proper understanding of the chromosome constitution of the polyploids. According to Lawrence, secondary association indicates allopolyploidy and he considers that the degree of such an association is a measure of the phylogenetic age of the form under examination. If we consider the  $F_1$  of a



species cross, the chromosomes of the two genomes A and  $A_1$  are often more or less differentiated but similar enough to permit loose pairing by means of chiasmata. After chromosome doubling, however, the true pairing will be autosyndetic A-A and  $A_1$ - $A_1$ , but due to the similarity between A and  $A_1$ , these chromosomes will still be attracted to each other. This attraction now results in secondary association. The degree of association is a measure of the similarity of the chromosomes. There is no material connection between the bivalents, and segregation is not in any way affected by the association. Secondary association arises at prometaphase, as at this stage suitable conditions exist for the approximation of the bivalents. Earlier the diakinetik repulsion keeps the bivalents away from one another. When once associated they remain so until interkinesis, when there is strong repulsion once again. At second prometaphase the members are again brought near each other. Secondary association is more marked at Metaphase II. This has been found to be so by Lawrence (1931), Müntzing (1933) and Nandi (1936). In *Calceolaria* also the association at the second division is more marked than at the first. If interkinesis is of short duration, the association may even survive until the second division. Secondary pairing is found mostly in plants with small chromosomes, as in plants with large chromosomes intimate association is difficult and there is little scope for free movement.

Catcheside (1937) concludes from a statistical analysis that secondary pairing is dependent upon the relative positions of bivalents at diakinesis, and the bivalents which happen to lie adjacent at diakinesis and which are capable of secondary pairing are so paired at metaphase. There is thus as much chance for maximum association as there is for no association on random distribution.

In *Pyrus* (Darlington and Moffett, 1930) as well as in the entire group *Pomoideae* (Moffett, 1931) the basic number is seventeen. On the evidence of secondary association the authors conclude that seventeen is a derived number as a result of multiplication of certain chromosomes in a basic set of seven. The number seventeen represents a case of successful aneuploidy involving a doubling of the whole genome and further reduplication of certain chromosomes. On the basis of this secondary balance, new chromosome multiples of seventeen have evolved. These observations have been criticised by Clausen (1921), Sax (1931) and Adati (1933).

Secondary association has now been studied in a large number of plants and good reviews are given by Lawrence, Müntzing (1933), Alam (1936), Nandi (1936), Iyengar (1939).

In the genus *Calceolaria*, the marked secondary association at

both the metaphases points to the fact that the cardinal number nine is a secondary number derived from presumably four. Lawrence (1931) investigated the cytology of *Verbascum phoeniceum* and found a low frequency of secondary association. The absence of multivalent associations led him to conclude that *Verbascum* is an old allopolyploid. But he does not mention the maximum grouping of the nine bivalents. The allopolyploid nature of the *Calceolaria* species studied is obvious from the marked secondary association. This conclusion finds strong support from the evidence of the relationship of the nucleolus to the somatic chromosome complement in some species. This aspect has been discussed elsewhere. It is significant that in *C. clibrani* where there is a marked secondary association at Metaphases I and II, indicating polyploidy, only one bivalent is attached to the nucleolus at prophase. Since polyploidy involves reduplication, one would naturally expect more than one bivalent in association with the nucleolus. But it is likely that the extra nucleolar chromosome has been lost, due to mutation or other causes.

Winge (1917) was the first to point out that the high frequency of multiples and the low frequency of primes among the gametic chromosome numbers in flowering plants might be taken to show that polyploidy was a common source of new species amongst them. It is now clear that a great number of plants are polyploids and that, of these, the majority of seed-bearing polyploids are allopolyploid, i.e. they have arisen (1) from hybridisation of species normally intersterile, by the functioning of diploid gametes, or (2) by somatic doubling of the chromosomes in sterile species-hybrids. Since fertility is an important factor in the survival of a seed-plant, natural selection will favour these new allopolyploids in which little or no allosyndesis occurs, multivalent association being inimical to high fertility. This differentiation of the chromosomes by structural changes will lead to bivalent pairing and result in a progressive evolution in the direction of diploidy, ultimately leading to the establishment of new diploid species with twice as many chromosomes as their progenitors. The ultimate consequence of progressive differentiation in the chromosomes is the establishment of species with a new basic number. In such cases secondary association is the only evidence of their duplicate origin.

Secondary pairing as a criterion of homology of the associated bivalents has been shown in a large number of plants, and has been used to determine the basic numbers of the species in haploids, diploids or polyploids. On this basis it has been shown that in rice, where the haploid number is twelve (Nandi, 1936) the basic number

is five. Similarly, in some of the apparent diploids reduplication of certain chromosomes has taken place, giving fertile species. These are thus secondarily balanced diploids, as in *Tricyrtis*  $n = 13$  and *Dicentra*  $n = 8$  (Matsuura, 1935) which are derived from basic sets of twelve and seven, respectively. In *Acer*  $n = 13$  (Meurman, 1933) the true basic number is twelve. A number of apparently diploid species are really secondarily balanced polyploids. Other cases are *Gossypium* (Davie, 1933), where  $2n = 26$  is derived from six or seven; *Brassica*,  $b = 6$  (Catcheside, 1937; Alam, 1936); *Solanum tuberosum* (Müntzing, 1933) where  $2n = 24$  is derived from  $b = 6$ ; *Cicer*  $b = 4$  (Iyengar, 1939).

In the diploid *Calceolarias* studied the maximum association at Metaphase I and II is  $3(2) + 1(3)$ , and the most frequent association is  $3(2) + 3(1)$ . The infrequent occurrence of multivalents indicates a definite drift towards functional diploidy. That structural changes in the chromosomes have taken place is indicated by the presence of inverted segments. Now, if we assume that four is the basic number, then the diploid number  $2n = 18$  is secondary. Assuming that the original constitution of the haploid set is A, gene mutation and structural differentiation would result in the formation of another set  $A_1$  from the original. A cross between A and  $A_1$  would be sterile due to failure of pairing. Amphidiploidy in the sterile hybrid would lead to doubling of the chromosome number, thus restoring fertility. Doubling in the hybrid may occur somatically, as in *Primula kewensis* (Newton and Pellew 1929) and *Nicotiana glauca* (Clausen, 1928) or gametically, as in *Raphano-Brassica* (Karpechenko, 1927, 1929). The amphidiploid would be fertile and constant through autotetraploidy. The general basic number in the Scrophulariaceae is eight. In the genus *Calceolaria* there is a reduplication of one pair of chromosomes in the course of evolution, due perhaps to irregularities in meiosis, thus giving  $2n = 18$ . A new basic number has been established which is a definite evolutionary step. Thus a new successful aneuploid series has arisen from the basic number four. The original diploid species are apparently extinct.

### *Calceolaria mexicana* Benth.

*C. mexicana* is native to South America and at the present day it grows luxuriously on hills in South India at altitudes varying from 4,000 to 6,000 ft. above sea level. The plant has admirably adapted itself to the climate of the tropical rain forest. Fyson (1915) has figured the plant in his flora of the Nilghiri and Pulney hill tops,

although in recent years doubts have been raised as to the identity of the species. This point has been discussed elsewhere (Srinath, 1939).

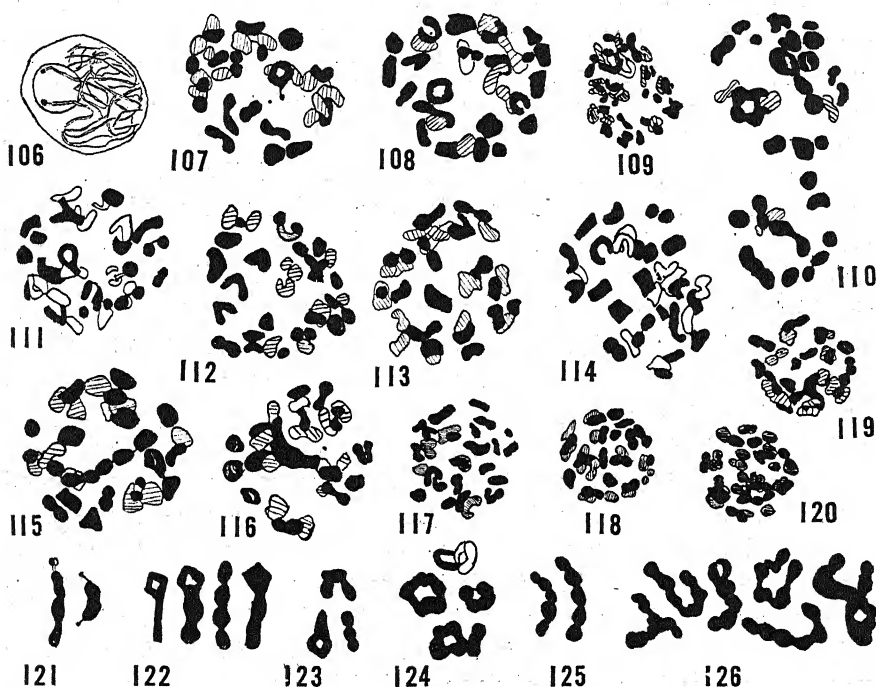
Material for the present study was collected during the summer months in India. Before fixing, the calyx and corolla lobes were clipped off and the bud then immersed into the fixative. Belling's formula for Navashin was used throughout. An exhaust pump was used to ensure quick fixation. The sections were stained according to Newton's iodine-gentian-violet schedule.

**Meiosis.** The somatic number could not be determined for want of root-tip material. Sugiura (1928), in his list of chromosome numbers of angiosperms, has figured a polar view of Metaphase I with thirty bivalents. Taking nine as the cardinal number, as is the case in the other species studied, this species has an aneuploid number.

Observations on meiosis were confined to diakinesis and later stages. A good polar view of Metaphase I was not available but a careful study of a number of cells at diakinesis showed thirty bivalents. In one cell at zygotene two bivalents were clearly connected to the nucleolus. (Fig. 106). Zygotene association was markedly uniform even in the region of the nucleolus: this was presumably due to the fusion of the nucleoli, as in this region the pairing of the chromosomes attached to the nucleoli is generally incomplete. This is due to the fact that when the nucleoli have not already fused, such chromosomes are delayed in coming together by having to pull their nucleoli after them. One pair of bivalents were attached to the nucleolus. (Fig. 107). The chromosome distribution of this stage was typical: that is, due to repulsion the majority of them are arranged on the surface of the nucleolus, with the rest distributed in the interior. (Fig. 108). A frequent association of chromosomes was observed in a large number of cells, the associations ranging from trivalents to a group of eight.

The bivalents were mostly rods with one terminal chiasma or rings with two. (Figs 109 and 110). Trivalents were arranged as chains, ring and rod (frying-pan) or Y. (Fig. 123). The quadrivalent types are illustrated in fig. 122. The chain quadrivalent was frequently observed. A chain of four chromosomes would need four chiasmata, while the other types of quadrivalents would have to have four chiasmata. Two chains of five are shown in fig. 125. Associations involving more than five chromosomes are illustrated in fig. 126. More than one multivalent association in a cell is shown in fig. 110. Here the configuration is  $24_{II} + 1_{IV} + 4 + 1_{III} + 1_I$ .

The occurrence of multivalent associations has been demonstrated in a number of plants. Meurman (1929) has described polyvalent associations of chromosomes in *Prunus laurocerasus* ( $n = 22$ ), a highly polyploid species; the associations ranged from trivalents to associations of seven, the trivalents being most common. In *Dahlia Merckii* (Lawrence, 1929) with thirty-six chromosomes, there are quadrivalents, two sexivalents and two octovalents, suggesting that the plant is a tetraploid with two of the eight chromosomes of the set still further reduplicated. In the pentaploid *Tulipa clusiana* (Newton and Darlington, 1929) all valencies up to the



Figs. 106-126. *C. mexicana*. 106. Early zygotene with two bivalents attached to the nucleolus. 107. Early diakinesis showing thirty bivalents. Two bivalents are satellited and are attached to the nucleolus. 108. Early diakinesis showing thirty bivalents. 109. Same as above.  $\times 3600$ . 110. Early diakinesis showing  $24_{II} + 1_{IV} + 4 + 1_{III} + 1_I$ . 111. Early diakinesis showing  $26_{II} + 2_{III} + 2_I$ . 112-114. Early diakinesis with thirty bivalents. 115. Early diakinesis showing a chain of five. 116. Early diakinesis showing an association of six chromosomes. 117-119. Early diakinesis showing thirty bivalents. In fig. 114 is an association of six chromosomes.  $\times 3600$ . 120. Early diakinesis with a ring of six chromosomes. 121. Two satellited chromosomes. The chromosome to the right is an ordinary satellited bivalent viewed from the top. 122. Types of quadrivalents. 123. Types of trivalents. 124. A ring of six and a ring of four. The third figure represents two interlocked bivalents. One bivalent is attached to the ring of four. 125. Two chains of five. 126. Different types of associations of chromosomes. The second figure has eight chromosomes in association.

quinquevalent have been observed. In quinquevalent, sexivalent and septavalent groups in *P. laurocerasus*, often three and sometimes four chromosomes were found connected to one chromosome, and Meurman suggests that the chromosome threads must have split prior to synizesis, the four ends of one chromosome being each attached to a different homologous chromosome.

The association of chromosomes in groups occurs very generally in autopolyploids. In a polyploid species like *P. laurocerasus* the chromosome complement is made up of a number of sets in which the chromosomes are so similar that they are all capable of pairing with one another. The presence of more than two homologous chromosomes explains the formation of such groups. There are also cases of allopolyploids which are derived by doubling the chromosome complement in a structural hybrid. In such heterozygotes where the chromosomes have undergone structural changes such as segmental interchange, pairing occurs between parts of structurally dissimilar chromosomes and larger associations are therefore possible. Such allopolyploids, as is the case with autopolyploids, have no differentiation between the corresponding chromosomes that would seriously interfere with their pairing at meiosis. High associations should then be possible, given segmental interchange in an ancestor and hybridity for the interchange in the plant. *Calceolaria mexicana* belongs to this class.

A ring of four chromosomes and a ring of six were observed and are illustrated in figs. 110 and 120. They have been drawn separately in fig. 124. A ring of four chromosomes can be explained in the basis of segmental interchange in non-homologous chromosomes of a hybrid. If two non-homologous chromosomes AB and CD exchange segments B and D, the heterozygotes resulting from this interchange would have the constitution AB, CD, DA, while the constitution of the original plant would be ABAB, CDCD. The former would give rise to a ring of four at diakinesis. Each chromosome in the ring may be represented as made up of two halves or segments which pair independently with homologous segments and are arranged differently in all the chromosomes of the ring. A ring of six implies two interchanges. It can be represented as AB, BC, CD, DE, EF, FA. This type of constitution is characteristic of an organism which is heterozygous in respect of the structural change which occurred in its ancestors. It is possible, however, that the length of two of the interchanged segments is not great enough for the frequent formation of chiasmata with homologous parts. The rings may then be replaced by a chain or several chains. (*Rhoeo discolor*, Darlington, 1929).

### Discussion

Chromosome ring formation was first observed by Cleland (1922) in *Oenothera*. The explanation of ring formation on the hypothesis of segmental interchange was first suggested by Belling (1925) to account for the semisterility previously observed by him (Belling, 1914) in *Stizolobium*. The semi-sterility was explained by him as due to certain non-viable chromosome combinations produced by the disjunction of the four members of the ring. Later, Belling and Blakeslee (1924, 1926) and Belling (1925) formulated the hypothesis of segmental interchange to interpret the peculiar configurations in secondary trisomics of *Datura*. Segmental interchange leading to ring formation has since been reported in *Zea* (Burnham, 1930; McClintock, 1931; Cooper and Brink, 1931), *Pisum* (Håkansson, 1929, 1931, 1934; Hammarlund and Håkansson, 1930; E. Richardson, 1929; Pellew and Sansome, 1931; E. Sansome, 1932), *Campanula* (Gairdner and Darlington, 1930), *Rhoeo*, *Tradescantia* and *Zebrina* (Darlington, 1921; Sax, 1931, Nebel, 1931), *Aucuba* (Meurman, 1929; Afify, 1933), *Drosophila* (Sturtevant and Dobzhansky, 1930; Muller, 1930), *Hypericum* (Hoar, 1931), *Circotettix* (Helwig, 1932).

On account of the peculiar morphology of its chromosomes, the study of a strain of maize known as "semisterile - 2" (Burnham, 1930; McClintock, 1931) provided the first complete proof of the correctness of Belling's hypothesis of segmental interchange. The two interchanged chromosomes could be distinguished on the basis of length, position and size of the region of attachment. In prophase the pairing of homologous portions of such chromosomes gives rise to a cross-shaped configuration of four, the centre of the cross marking the point of interchange in two of the chromosomes. In late prophase the four open out, remain attached at their ends and shorten to form a ring at diakinesis and metaphase. Hybrids with a ring of four have been obtained by crossing different homozygous races (*Pisum*, Håkansson, 1929, 1931, 1934; *Datura*, Blakeslee, 1928, 1929). Again, hybrids with a ring of six or two rings of four have been produced by crossing two parents, which were heterozygous for two interchanges (*Datura*) or by crossing two races differing in one interchange and having one gametic type in common (*Pisum*, Sansome, 1932; *Oenothera*, Gates and Catcheside, 1931; and *Campanula*, Gairdner and Darlington, 1931). Ring forming types have also been obtained by X-radiation in maize, *Nicotiana*, *Oenothera* and rice.

In nature ring-forming types arise as a result of the union of

dissimilar gametes, the dissimilarity being due to the interchange taking place in an ancestor. The interchange may have given rise to the heterozygote at once in nature or a new homozygous race may have formed which later gave rise to the hybrid by crossing. An important question, bound up with such changes, is the survival value of structural changes in the chromosome and the part they play in evolution. It is clear that the heterozygous state is not advantageous to the plant, in so far as it results in sterility brought about by irregular segregation and the production of non-viable gametes. This has been verified in artificially produced interchange heterozygotes in Maize, Rice and Nicotiana. But in *Triticum* and *Datura* (Blakeslee and Cleland, 1930) sterility in hybrids was produced presumably because of the failure of orientation of the ring. Here probably the parental complements are recovered in a large proportion of cases. Segregation would lead to the production of homozygous and heterozygous types. *Campanula* and *Aucuba* may be said to represent a middle stage as they show the persistence of partial catenation in nature. It is also possible to say that they represent a stage in the natural evolution of the ring, resulting in the perfect permanent heterozygotic condition found in *Oenothera*. The true breeding potentialities in this genus depend upon a "balanced lethal mechanism," as suggested by Muller (1918). This hypothesis assumes that gametes of neither complex (accepting the complex-heterozygote hypothesis of Renner (1925) that the two kinds of gametes have different genetic complexes) can yield viable homozygotes.

The arrangement of multiple bodies on a bipolar spindle is *ipso facto* irregular. It is reasonable to expect a certain degree of irregular segregation in polyploid species with frequent multivalent associations. But in spite of the high associations in *Prunus lauro-cerasus*, Meurman found that the segregation was remarkably regular. It must, however, be recognised that in high polyploids the basic complement is reduplicated so many times that a little irregularity would not seriously interfere with the production of viable gametes. Kobel (1923) found that in varieties of *Prunus lauro-cerasus* the somatic number varied widely in different individuals. From this he concludes that the chromosome number by itself is not necessarily of importance for the viability of the zygote. In several saxifrage species the chromosome number is very unstable, (Skovsted, 1934) and in *S. granulata* the somatic number was found to vary between forty and sixty without any morphological effect. For this reason Skovsted concludes that the vital genes must be repeated so many times that one chromosome more or less does not



affect the physiological balance. In *C. mexicana* the anaphases are perfectly normal in spite of the frequent multivalent associations.

It was pointed out earlier that the chromosome number in *C. mexicana* is aneuploid compared to the multiples of nine which characterise the other species. That this number has been derived from a tetraploid seems certain. The association of bivalents with the nucleolus points to its tetraploid ancestry. Calceolarias are mainly inhabitants of the South American Andes where the climate is wet and temperate and they grow mostly between attitudes of 6,000 and 14,000 ft. above sea level. While there is no doubt that *C. mexicana* has been introduced into India, the present day distribution there suggests that a type has been evolved which has adapted itself to the peculiar climatic conditions of the tropics. It is suggested that the original tetraploid due to geographical isolation and changed environmental conditions doubled itself to give rise to an octoploid with  $2n = 72$  chromosomes. When once an octoploid was formed, the chromosome balance might not have been proper to enable it to survive. As a result, probably one genome was lost. The loss of sets of chromosomes in a polyploid species giving rise to lower polyploids is a process occurring in *Rosa* (Hurst, 1929). The elimination of chromosomes presumably enables the species to exist in nature by securing a proper chromosome balance. Other meiotic irregularities may have been responsible for the loss of three more chromosomes giving a stable species with sixty chromosomes. It is natural to infer that the other nucleolar chromosomes have been involved in this process of chromosome elimination. The high multivalent associations are intelligible in the light of the fact that the basic complement has been duplicated so many times. The original tetraploid and the octoploid have obviously been eliminated in competition with the more successful species evolved. The occurrence of rings in a natural species adds another piece of evidence to the conception that structural changes in chromosomes have a survival value and play an important part in the evolution of species in nature. While the other species of *Calceolaria* are in multiples of nine, this species has established a new basic number which is a multiple of ten.

### Summary

(1) Meiosis is described in three diploid and one aneuploid species of *Calceolaria*. The diploids are *C. elibrari* ( $2n=18$ ), *C. dentata* ( $2n=18$ ) and *C. Banksii* ( $2n=18$ ). The aneuploid species, *C. mexicana*, has  $2n=60$ .

(2) In the diploids, one bivalent is attached to the nucleolus at

diplotene and diakinesis, while two bivalents are attached to the nucleolus at zygotene and diakinesis in *C. mexicana*.

(3) At diplotene in the diploids, interstitial chiasmata persist in 35% of the cases but are terminalised at metaphase. Multivalents are rare. In the aneuploid species associations of chromosomes ranging from trivalents to groups of eight are described. Rings and chains of four and six chromosomes were also found.

(4) An analysis of secondary association in the diploids at Metaphase I and II shows a maximum grouping of 3(2) + 1(3). The diploid number  $2n = 18$  is, therefore, considered to have been derived from the basic number 4, indicating allopolyploidy.

(5) In the diploids the presence of inverted segments is indicated by the formation of chromatin bridges at Anaphase I.

(6) The nature and significance of ring formations in the aneuploid species, the value of structural changes in evolution and the origin of the aneuploid species have been discussed.

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## Behaviour of Chromonemata in Mitosis

### IX. On the configurations assumed by the spiralized chromonemata<sup>1)</sup>

By

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The studies of chromosomes by means of the polarization microscope seem to show that the chromonemata in the chromosome assume one configuration in a certain method of fixation and another configuration in another method (BECKER and KOZBIAL, 1937). In his critical review of chromosome structure<sup>2)</sup>, KUWADA (1939) has postulated a hypothesis of chromosome cycle in which it is assumed that there are three different configurations taken up by the chromonemata in the chromosome. The fine and coarse structures, experimentally shown by SINKE to exist in the nucleus and observed by NAKAZAWA in living material (both with KUWADA, 1939), are those structures which led him to this assumption. In this hypothesis it is assumed that the fine structure is due to the separation of the spiralized chromonemata in each chromosome from one another so as to present a fine but complex appearance in the nucleus; and the coarse structure due to a union being made between the two sister chromonemata of the separated four, the number of the chromonemata for one chromosome which NEBEL believes to be the actual one (cf. KUWADA, SINKE and NAKAZAWA, 1939, foot-note on p. 397). Looking back into the literature on telophasic chromosomes, KUWADA has further assumed that there should exist two types in the coarse structure. In one type, the united chromonemata take up the spiral or S configuration, and in the other the twisted or T configuration, the form which an orthospiral (KUWADA, 1939) can assume when drawn out. It has been also assumed that the twisted configuration is due to the chromosomes being in a more dehydrated condition than in the spiral configuration. It is the main object of the present

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1) Investigation supported in part by grant from the Science Research Fund of the Department of Education.

2) In this critical review the authors' names, AISIMA under Fig. 5 on p. 222 and SINKE under Figs. 10 and 11 on p. 237, were left out by mistake, and are to be put respectively in place, each preceded by "After" and with parentheses. *Galtonia* in the 15th line from the bottom on p. 232 is also to be read for *Gasteria*. This opportunity has been taken to make these corrections.—Y.K.

investigation to see whether these hypothetical configurations of the chromonemata really exist in the resting nucleus or not.

### Observation

SINKE (l.c.) has demonstrated that a coarse structure of the nucleus results from the nucleus of a fine structure by dehydrating it by means of hypertonic solutions. This suggests that a nucleus of the coarse structure will be transformed into one of a fine structure if it is put in a hypotonic medium. If, therefore, the two types of the coarse structure, one with the S configuration and the other with the T configuration, really exist, it is to be expected that a similar experiment, or the observation with a hypotonic solution as medium, will throw some light on the genetic interrelationship assumed between these two types of the structure. From this point of view, not only liquid paraffin but also tap water were used as mounting media in the following observations, staminate hairs of *Tradescantia reflexa* being used as material.

1. *Telophase.* In our previous observation with liquid paraffin, the main results of which were published in the "Behaviour" III (1934), we often noticed a structure in which the chromonemata appear as a series of dots, like a broken line, two such being found parallel or twisted about each other in some measure in the chromosome. In the preparation fixed with acetic acid, the anaphasic chromosomes also appeared to consist of two chromonemata with dotted or knotted appearance (Fig. 1). Spiral turns were also observed more or less clearly in living nuclei in telophase, and it was doubted at that time whether the structure of dotted appearance does not represent an optical section of a spiral.

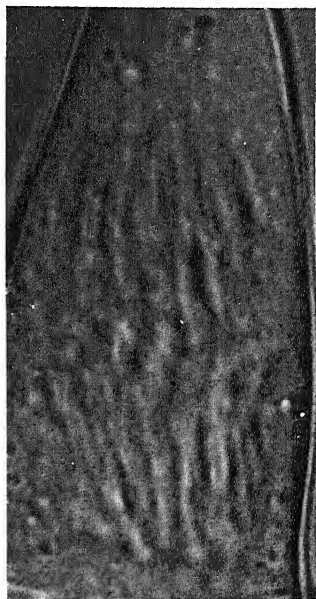


Fig. 1. Chromosomes in anaphase, fixed with acetic acid. Zeiss' apochr. obj. 1.5 mm  $\times$  comp. oc. K. 12. Figs. 2-3, 5-8, 10-11: Zeiss' apochr. obj. 2 mm  $\times$  comp. oc. K. 12.

Now it seems to us highly probable that the two strands with knotted appearance are not the chromonemata, but the chromatids which may contain two chromonemata in each (KUWADA, 1939). The chromatids may run parallel or be twisted about each other to

some variable extent. Recently SINKE<sup>1)</sup> has observed telophasic chromosome transformation with the same material using liquid paraffin as medium, and has reached the same conclusion, i.e. that in the telophase each chromosome may contain two strands mostly with the knotted appearance. In the renewed observation with liquid paraffin we also obtained the same results, and had an impression that the twisting between the chromatids becomes greatly reduced during the telophasic transformation in this material. In the early telophase, the matrix change being incomplete as yet, individual chromosomes are distinguishable from one another (Fig. 2a), but in the late telophase where the matrix change has greatly

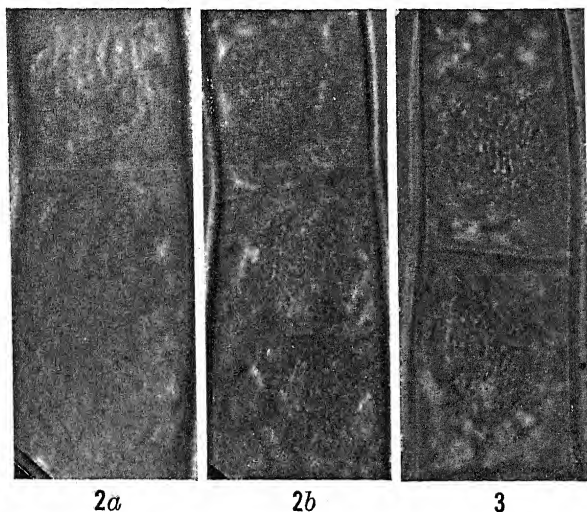


Fig. 2. Telophase, mounted with liquid paraffin. a. Early telophase showing chromosome distribution (1.40 p.m.). b. Late telophase showing chromatid distribution (3.21 p.m.). At 1.52, the chromosomes showed some internal structure, but the chromosome distribution was still clear. At 2.12, the nuclear structure was no longer of the chromosome distribution, but of the chromatid distribution just as shown in b. Fig. 3. Telophase at 3.28 p.m., showing chromatid distribution. Mounted with tap water at 2.10 p.m.

progressed, the chromatids become uniformly distributed, and it is hardly possible to distinguish which two chromatids form the pair belonging to one chromosome, though the straight arrangement is still clearly maintained (Fig. 2b). The chromatids in this case present the knotted appearance, though the spiral configuration may also occur. In the observation with water as medium, we find also the knotted appearance of the chromatids (Fig. 3).

When the nucleus enters into the interphase the straight strands of the knotted appearance become partly wound, so that the straight arrangement in the telophase is lost, and the nucleus presents a diffuse structure. A closer observation and an inspection of the photomicrographs of these nuclei show that in both media, water

1) Unpublished.



and liquid paraffin, the strands are no longer of a mere knotted or dotted appearance throughout, but in part are coiled into irregular spirals such as those which we can obtain when a tightly twisted and stretched thread with fixed ends is loosened merely by approximating the two ends without releasing them or untwisting the thread. These spirals are of relatively long pitch and small diameter, of variable length, having an irregularly drawn out appearance (Fig. 4). This structure seems to explain one reason why the straight strands in the telophase look wound in the interphase. It seems that in anaphase the chromosomes are of a highly dehydrated state, and hence the chromonemata contained in them must also be in the same condition, so that they are forced to transform their spiral configuration into a tightly coiled, DS, or twisted, DT, configuration such as shown in Fig. 9, through their shortening and hence thickening as the result of dehydration. These configurations, especially the DT, will appear to be "dotted" or "knotted" in a superficial view. In the anaphase this structure is not visible in the living state on account of the dense, highly dehydrated matrix of the chromosome, but in the telophase the nucleus becomes gradually hydrated and the matrix substance undergoes the change that enables us to see the chromatids with this dotted appearance clearly. In the interphase the chromatids become hydrated to a certain extent, and the tightly coiled spirals with the dotted appearance are partly loosened to variable extents, to present corresponding spiral configurations of variable pitches and diameters, so that the whole chromatids appear to be wound irregularly. This is the TS configuration, an intermediate type between the T and the S. The nuclei of this structure present a diffuse appearance.

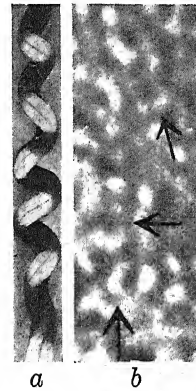


Fig. 4. *a*. Diagram of TS configuration with a turning point, in the nucleus of diffuse structure, showing light part presenting dotted appearance of the whole structure. Comp. Fig. 9, TS. *b*. An enlargement of a part of the resting nucleus of a *Tradescantia* staminate hair cell from the original plate of Mr. NAKAZAWA which has been reproduced in Fig. 9 *b* of KUWADA, 1939. Comp. the chromatids indicated by vertical arrows with *a*. The horizontal arrow indicates a turning point.

In the telophase the chromonemata may also present the spiral configuration (Fig. 5), in which case the anaphasic configuration should also be one maintaining the spiral configuration, DS. In the case of such a telophase the spirality may be more conspicuous also in the early stage of interphase than in the case where the knotted appearance is predominant in the telophase. This spiral

configuration of the interphasic nucleus may be designated as CS in view of the fact that the nucleus is of a coarse structure. In this case, the chromosome territories are perceptible and the nucleus

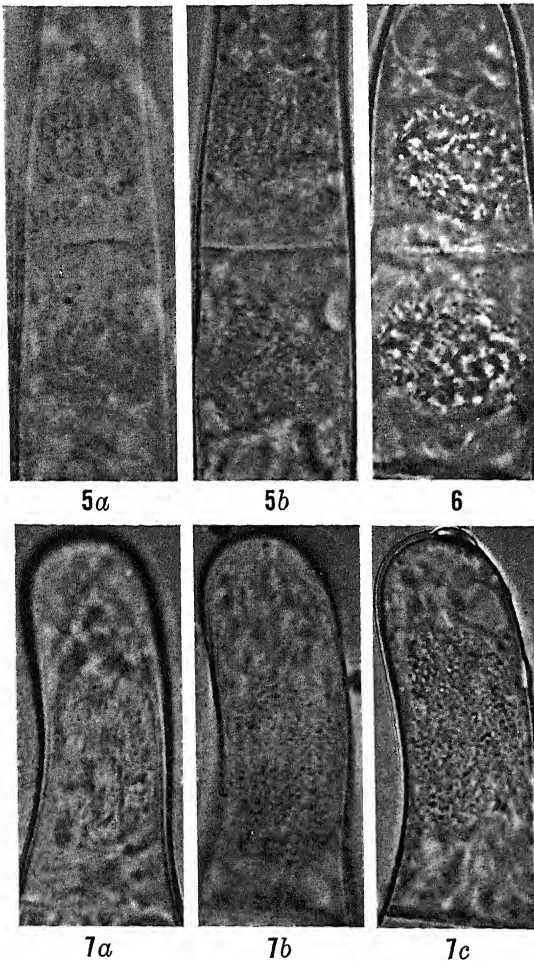


Fig. 5. Telophase. *a*, photographed at 11.26 a.m. *b*, at 11.41 a.m., showing spiral configuration. Mounted with tap water at 11.10 a.m. The spiral configuration in telophase is also observed in the preparation in which liquid paraffin is used as mounting medium. Fig. 6. Telophase, photographed at 6.52 p.m. Mounted with water at 1.50 p.m. Fig. 7. *a*, Resting nucleus of the coarse structure of twisted configuration, at 1.57 p.m. *b*, The same nucleus at 4.00 p.m. Spiral configuration is manifest, transverse striation being marked. *c*, The same at 5.10 p.m., showing a finer structure in which a reticulate appearance of the chromosomes is perceptible. Mounted with water at 1.50 p.m.

presents an aggregated appearance. This is the characteristic feature of the nucleus with the spiral configuration as contrasted with the nucleus with the TS configuration, which presents a general diffuse appearance. Strictly speaking, any one nucleus is not of the pure type of one structure, but of a mixed type in which one structure predominates over the others.

In one instance a peculiar case was observed. Hairs were mounted with water at 1.50 p.m. Some hours later a metaphase figure was found. In this metaphase the chromosomes were very thick and short, and appeared homogeneous in structure, being extraordinarily swollen. We assumed that the cell was in a very unhealthy condition and the mitosis would not proceed further. To our astonishment, however, it was found later that the mitosis had proceeded to telophase. The nuclei were photo-

graphed immediately (at 6.50 p.m.) and the observation was continued up to 7.50 p.m. During this period, little change was noticed in the structure of the nuclei, suggesting that the mitosis proceeded extraordinarily slowly. As seen from Fig. 6, it seems that the matrix change is as yet incomplete in these stages. This incompleteness in change of the matrix prevents accurate observation, but the chromosome structure appears to be finer than in the ordinary telophasic nucleus. From this case of incomplete matrix change, we are impressed with a suspicion that the anastomoses between chromosomes observed by some authors in living material may perhaps represent the parts of the matrix not yet undergoing the complete change (ABRAHAM, 1939).

2. *Resting stage.* Resting nuclei in the hairs mounted with water were watched under the microscope for several hours to see if any change in the structure of the nuclei is brought about in the water medium. So far as our observation goes, in only one case a remarkable change was observed with certainty. Fig. 7a is the photomicrograph of the nucleus showing its original structure, taken soon after it was mounted with water. In this nucleus, as will be seen from the photomicrograph, the knotted or dotted appearance (DT) predominates over the spiral appearance, and the general straight arrangement of the chromosomes is pronounced. In *b* which was photographed after about two hours, the straight arrangement is still maintained, but here the knotted appearance has disappeared and the spirality is rendered more clearly visible. This configuration represents the CS. In *c* which was photographed about one hour later still, the general structure is much finer than in *b*. This structure resembles very much that of the natural nucleus photographed by NAKAZAWA (Fig. 3 of KUWADA, SINKE and NAKAZAWA, 1939) which we have called the nucleus of fine structure, consisting of chromosomes having the structure of a reticulate appearance and showing the general aggregate appearance. We may probably say that by this observation it is demonstrated that in water medium the DT configuration can be transformed into the CS, and further into at least a fine structure (comp. Fig. 9). The reasons which can be suggested as to why experimentally such a transformation of the nuclear structures in water medium as that mentioned above was possible only in so extremely rare a case may probably be as follows:—

1) From the fact which he observed (SINKE, 1939) that while in animal cells a heterogeneous nucleus is transformed into a hydrated nucleus when treated with water or a hypotonic solution, such a transformation does not take place in plant cells, the entrance

of water being prevented by the cell wall pressure, SINKE<sup>1)</sup> is inclined to the view that when the cells are treated previously with a hypertonic solution, this transformation becomes possible also in the plant cells, probably because of the fact that the cells undergo a certain change in their permeability as a result of plasmolysis. It seems probable that in our case, in which the transformations in water medium of the nuclear structure from one to another were observed, the cell had undergone some exceptional change in its permeability during manipulation.

2) It may also be considered that some internal change must necessarily take place in order that the nucleus become one of fine structure, if in this structure, which may be designated as FS, it is the characteristic feature that the chromonemata of spiral configuration in the chromatid, presumably two in number, are separate from each other. This point seems to be very important, because the typical fine structure appears to be of rare occurrence. The

DT structure of the nucleus with which we observed the transformation into a fine structure might have been a secondary structure derived from FS, if the fine structure into which we could transform the DT structure is really one to be represented by FS. Fig. 8 shows a case of this secondary transformation of the fine structure into a coarse structure, by dehydration. In *a*, the nucleus shows a reticulate structure in the lower half, which is delimitable from the upper half by an oblique line passing through the center of the nucleus

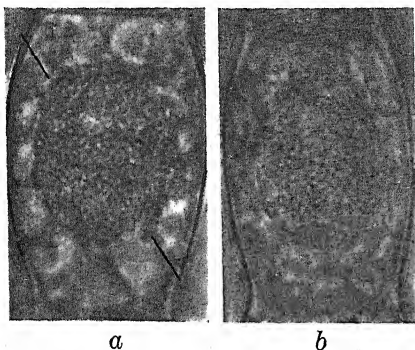


Fig. 8. *a*. Resting nucleus mounted with water at 11.05 a.m. and photographed at 11.18. The mounting medium was replaced by 0.1M cane sugar solution at 11.30, and again by 1/4 M at 1.45 p.m. *b*. The same nucleus photographed at 2.35 p.m.

from upper left to lower right; and it also shows the coarse structure of T or TS configuration in the upper half, which is especially pronounced near the center. In *b* which was photographed after being dehydrated gradually by replacing the water medium with cane sugar solutions of graded concentrations not strongly hypertonic, the whole nucleus shows the coarse structure except the region near the periphery on the left hand side where the structure appears somewhat finer.

1) Verbal information.

SINKE<sup>1)</sup> has demonstrated that when a nucleus (staminate hair) of diffuse structure (TS) is put under the influence of high temperature, the structure is transformed into a coarser structure (probably DT), and when the hair is put back in the room temperature, this coarse structure changes gradually until it attains the structure of the CS configuration. It has been also demonstrated by the same author (1939, with KUWADA and NAKAZAWA) that a coarse structure (probably DT) brought about by dehydration of the nucleus of a fine structure is brought back to the original fine structure by hydration (comp. also the experiment by SINKE shown in Fig. 10 of KUWADA, 1939, in which *a* shows the original spiral configuration, and *b* shows the diffuse structure (TS) produced from *a* by dehydration).

From what is mentioned above, we may perhaps be able to say that the configurations, S and T, are mutually transformable. The configurations DT, DS, TS, CS and FS which we can presume at present are diagrammatically shown in Fig. 9. In these cases, it is assumed that the chromosome consists of two chromatids, each containing two chromonemata forming a double-stranded spiral. In the configurations DT, DS, TS, and CS, the two chromonemata

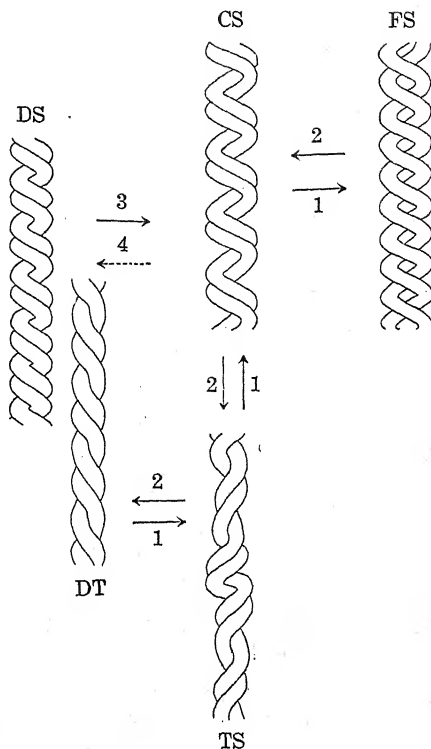


Fig. 9. Diagrams showing chromonema configurations, DS, DT, TS, CS and FS, on the supposition that the chromonemata in the chromatids, of which only one of the two in the chromosome is illustrated in each case, form double-stranded spirals (case IIIa in Fig. 12). Arrows show mutual transformability of the configurations. The transformation through route 1 is exemplified by Fig. 7, and that through route 2 by Fig. 8 and SINKE's experiments with hypertonic solution. The heating experiments of the same author also show that the transformation can take place through route 2 when material is heated, and through route 1 when it is cooled. These reversible transformations have experimentally been shown also by this author by treating material first with a hypertonic solution and then with a hypotonic solution or water. For route 3, compare Fig. 5. Route 4 may exist, but it is hardly possible practically to distinguish between DS and DT.

1) To be published later.

are united together to form a double thread and assume the twisted configuration T or the spiral configuration S, or an intermediate configuration TS; and in the FS configuration they are separated to form a "relational spiral". The nuclei in which the two chromonemata are united together are collectively called of coarse structure, and those in which they are separated are of fine structure which is designated by the letter F. The DT and DS are dehydrated configurations and the FS is a hydrated configuration in which the hydration is connected mainly with the matrix substance. The CS structure, which is also a hydrated configuration as contrasted with the DT and DS, can be further hydrated as a whole without being transformed into FS, or in other words, with the sister chromonemata remaining united together as they are in the CS, so that the whole structure may become less clearly visible. The CS structure, especially this hydrated CS which may be designated as HS very much resembles in appearance the FS, and it is often very difficult to distinguish between them.

The interpretation made above of the nucleus of the diffuse structure as consisting of the chromatids of twisted or irregular spiral configuration is supported by the fact that there is often a close resemblance in general appearance between the nucleus of this structure and the nucleus in the mid-prophase after the spiral stage, where the old spirals are being drawn out. There is of course a great difference in the thickness of the threads, but the resemblance is so remarkable that one might mistake the prophasic nucleus for a resting nucleus, if he were shown a photomicrograph of this nucleus alone.

If a nucleus of fine structure presents the diffuse appearance, this fine structure will be one which is to be represented by the symbol FT or FTS. If such nuclei are demonstrated to exist, it will be critical evidence to show that each chromonema is spiralized independently from others, because if a pair of chromonemata forms together a double stranded spiral, the two chromonemata cannot be separated from each other to present the fine structure F, when the spiral takes the drawn out form, being forced to form a strand of two threads twisted on each other like the DT configuration; hence the fine structure FT or FTS cannot exist in this case. Since no such single nucleus, clearly of the fine structure presenting the general diffuse appearance, has as yet been found, the various coarse structures are provisionally assumed to be derived from the structure FS as illustrated in Fig. 9, though it has not yet been determined with certainty whether this FS structure really exists or not.

In most cases where we observed the fine structure, it is the characteristic feature that the general straight arrangement is perceptible. The straight arrangement involves the perceptibility of the chromosome territories. The number of these territories shown in the median optical section of the apical cell nucleus of this material roughly corresponds with that of the chromosomes of the *virginica* staminate hair nucleus illustrated by BĚLAŘ (comp. his Fig. 2b with our Fig. 7c). In the case of the nuclei of the spherical shape, the straight arrangement does not generally exist, but the chromosome territories are perceptible just to the same extent as in the case where the straight arrangement is visible. The straight arrangement concerns only the chromosome arrangement, and is not of primary importance in respect of the nuclear structure. The condition which is of important significance is that the chromosome territories are perceptible and the nucleus presents an aggregate appearance. This condition is brought about by the retention of the spiral configuration, hence the chromosome territories are visible in any case where the spiral configuration is retained, and especially pronounced in the hydrated configurations HS and FS. This situation makes it very difficult to distinguish these two structures with fine appearance, although the FS structure is characterized by the reticulate appearance which is presented within each territory. The fact that the hydrated structures present the spiral configuration seems to show that the chromonemata of a straightened form such as the T configuration have a strong tendency to resume the spiral configuration, if there is no impediment. This means not necessarily that the chromonema spirals are "constructional" (MANTON, 1939), because the spirals can be drawn out when the matrix substance is affected by some artificial means (cf. MIMURA's experiment shown in Fig. 2 of KUWADA, 1939), but suggests that they are such spirals, for instance, as those which forcibly twisted threads can assume when they, in a confined space, strive to return to their original untwisted condition without rotation (KUWADA, 1937). If these spirals are forcibly brought to take a straightened form, and if in this condition, moreover, untwisting is not allowed, a great tendency of retaking the spiral configuration must be exerted. It seems that in bringing the chromonema spiral into this condition the contraction of the matrix plays a great rôle. In the interphase the chromosome matrix is in a swollen state, and we must here then probably assume a matrix of another kind—the chromatid matrix. In the case of the typical DT too, the straight arrangement may be apparent in the nucleus, if the telophasic arrangement has not been

greatly disturbed. These nuclei show, however, a diffuse structure of some kind, since the spirality is here concealed.

The chromosome territories become also visible when each chromosome contracts. In one case we observed that the nuclei of a hair showed the chromosome contraction to the extent that each territory was clearly visible, probably being in an unhealthy condition due to certain ill-treatment during manipulation. We were interested in this appearance of the nuclei, and preparing for photomicrographing, but when ready, it was found that the nuclei presented the diffuse appearance as beautiful as we see in the healthy nuclei, without showing any perceptible sign of the chromosome aggregation. This fact may be regarded as disproving the view of the nuclear structure as one system of colloid whether the system be simple or compound. It must show that each chromosome system is maintained in the nucleus without mingling, even though it appears superficially to be nothing more than one diffuse structure. The chromosome individuality must be maintained in the resting stage (cf. FUJII and YASUI, 1935).

3. *Prophase.* The mitosis may proceed normally in the water medium, but very often a change was found (cf. WADA, 1932, SIGENAGA, 1937) to take place, probably in the matrix substance, as a result of the abnormality in water relation in the cell, at a certain definite stage in most cases, which probably corresponds with the stage shown in Fig. 2e of BĚLAŘ (1929). At first, the spirality becomes more clearly visible in the chromosomes than in the corresponding stage in the normal mitosis (Fig. 10c, d). The threads of the spirals are thin, and the nucleus appears at first sight to be of a fine structure (Fig. 10e). The change proceeds further, and the nucleus is finally transformed to the structure which resembles the diffuse structure of the resting nucleus (Fig. 10f). The threads are now thicker than in d and e. The spirality is much less conspicuous in this stage, and it appears that the spirals in the earlier stage have been drawn out. It seems that this drawing out is connected with thickening of the threads which is in turn, it seems highly probable, connected with the growth of the divided chromonemata. If this interpretation is correct, it leads us to the conclusion that the splitting of the chromonemata must have taken place in an early prophase, hence the resulting nucleus is di-diploid. This nucleus is about as large as when it was in the spiral stage. The comparison of the nuclear sizes as a support of this conclusion should, however, be made between the nuclei at corresponding stages. The nucleus in question corresponds in stage with young interphasic nuclei produced through the normal mitotic processes. These interphasic



nuclei are much smaller than the nucleus which we are presuming to be di-diploid. If the phenomenon of the prophasic nucleus proceeding back to the resting stage is correctly interpreted above, we may regard this katachromatic change taking place abnormally in the prophase, as furnishing a mechanism of WINGE's "indirect chromosome binding"

(KUWADA, 1939).

In this connection, it is a very interesting fact that according to MC CLENDON<sup>1)</sup> dividing eggs are much more sensitive in plasmolytic experiments than unfertilized eggs. It seems not improbable that in interspecific hybrids in which there may be expected a certain disturbance in the normal physiological equilibrium to be established in the homozygous state (KUWADA, 1928), this sensitivity may be so great as to change the normal water relation in the cell and to result in the abnormality that in

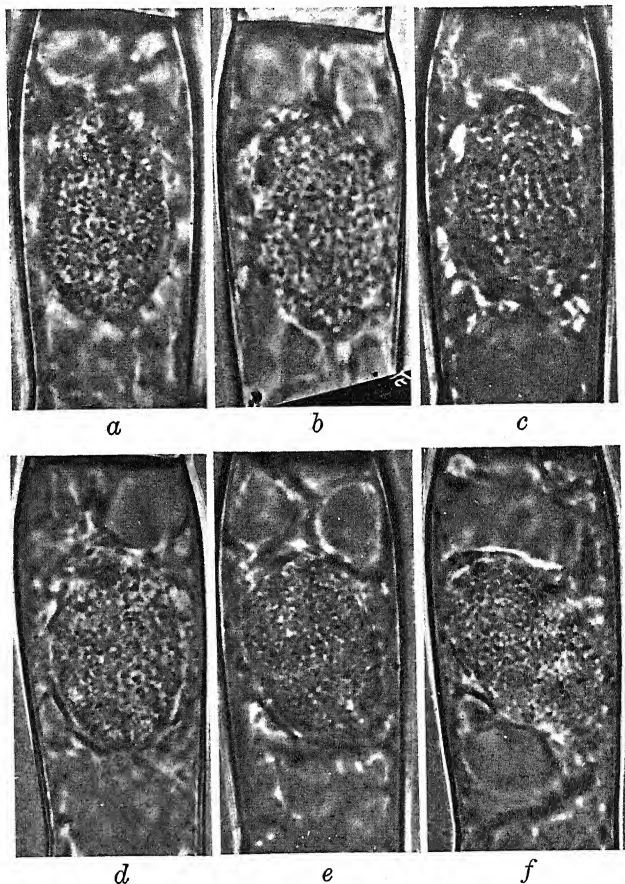


Fig. 10. Abbreviated mitosis experimentally induced. *a.* Spiral stage at 1.25 p.m. *b.* at 1.45 p.m. *c.* at 2.35 p.m. *d.* at 2.55 p.m. *e.* at 3.35 p.m. *f.* 5.10 p.m. Mounted with water at 1.15 p.m.

the first division after fertilization the anachromatic process is extremely abbreviated, or at least the katachromatic process takes place before the mechanism of polar separation of daughter chromosome comes into action.

This abbreviated mitosis artificially induced may be regarded

1) Cited from SPEK (1920).

as an experimental demonstration of endomitosis (GEITLER, 1939). In both cases, the chromosome separation mechanism having lost its existence, they are essentially the same. The distinction between the two is only in the manner of occurrence and in the degree of chromosome development, one occurring occasionally as an abnormal case without full development of chromosomes and the other successively mitosis after mitosis with fully developed chromosomes; as a response to a certain temporary condition in the former case and to a continuous one in the latter, that is peculiar to the tissues in which the mitosis (endomitosis) occurs.

It may be expected that in these mitoses lacking the chromosome separation mechanism, especially those with incomplete chromosome development, sister chromosomes are intimately connected with each other, so that they may appear in the succeeding mitosis in pairs (LORZ, 1937; GENTCHEFF and GUSTAFSSON, 1939). BERGER (1937) has observed this pairing or grouping in a mosquito in which the endomitosis takes place during the larval period.

The case of myxoploidy which is generally explained as occurring as a result of two successive chromosome splittings taking place in one mitosis (case 2c of MIDUNO, 1938) should be explained as in reality two successive mitoses of which the first one is an abbreviated mitosis, because in mitosis the chromosome splitting is a more essential phenomenon than the chromosome separation, hence what should represent a mitosis would be the former rather than the latter. This conclusion is verified by the discovery of endomitosis. The fact of the "uniform nuclear control" of the chromosome reproduction, that is, the fact that the chromosome number increases in the series 2<sup>n</sup> is also more simply explainable by the occurrence of abbreviated mitosis than by a mere assumption that it is brought about by "internal reproductions" (GENTCHEFF and GUSTAFSSON, 1939).

In one instance a very instructive case was met with (Fig. 11). The hairs were mounted with water at 10.00 a.m., and a resting nucleus of a diffuse structure (*a*) was watched under the microscope to see what change of the nucleus structure might occur under the influence of the water medium. About one hour later it was found unexpectedly that the nucleus was in the spiral stage (*b*). About 3 hours and a half after this, the nucleus proceeded to a very late prophase (*e*) passing the spireme stages (*c*, *d*) as in the normal mitosis. In the earlier spireme stage the nuclear threads appeared solid and single (*c*), but in the later spireme duality was visible in some of the threads (*d*). In the late prophase (*e*) the chromosomes were no longer of the solid structure, but showed a certain hetero-

geneity which suggests the internal spiral structure. Twenty minutes after this stage the chromosomes were all of a fine spiral structure (*f*), and after another 20 minutes the spiral structure was more evident than before (*g*). Then a pressure was given unintentionally. The nucleus shrank suddenly, and the chromosomes also shrank and became highly refractive (*h*). The observation was continued further for 3 hours and a half. The change was irreversible.

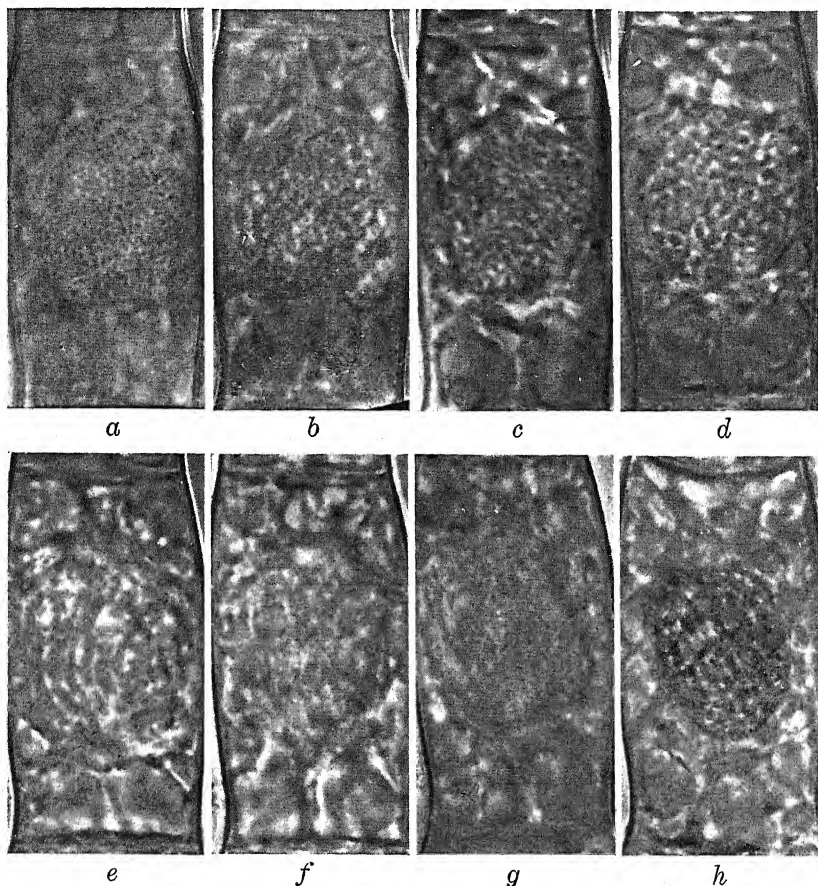


Fig. 11. Abbreviated mitosis with anachromasis proceeding further than in the case shown in Fig. 10. *a*. Resting stage at 10.15 a.m. *b*. Spiral stage at 11.15. *c*. Spireme stage, 12.10 p.m. *d*. 1.20. *e*. 2.40. *f*. 3.00. *g*. 3.20. At 3.35 a pressure was given unintentionally. *h*. 3.37. Mounted with water at 10.00 a.m.

Though in this case it was not possible to trace the further change of the nucleus, we have two important results. 1) The nucleus of the coarse structure directly enters into mitosis. Since most nuclei are of the coarse structure especially of diffuse appear-

ance, the first visible sign of mitosis in general must be a disturbance in the uniform distribution of the chromonemata, or the contraction of the chromosomes, which is immediately followed by a nuclear hydration as a step towards the spiral stage, as illustrated by the photomicrographs of NAKAZAWA (l.c.). 2) The chromosomes in prophase appear to be solid, but are in reality of the spiralized structure. The spiral is a very fine one with a far greater number of turns than the number which we can estimate in anaphase. This must be borne in mind in the study of the behaviour of chromonemata in mitosis. The number of turns must be reduced as the chromosomes become short and thicker. If, however, anaphasic chromosomes show chromatic spirals with comparatively too small a number of turns, it should be quite possible to assume that they are chromatid spirals.

In one case, a nucleus which appeared to be in the spiral stage was rendered in water medium of an apparently fine structure about 5 hours after being mounted, without showing any development in stage. The preceding stage was not observed, and it is open to question whether the nucleus was really in the spiral stage or not. This case is left for a further investigation.

### Discussion

In the present investigation the following points were confirmed:—

1. In telophase, the results of observation with water medium coincide with those obtained with liquid paraffin in most cases.
2. Both knotted or dotted and spiral configurations of chromonemata exist in the telophase.
3. The knotted configuration in the resting stage can be transformed into the spiral configuration, and further into a fine structure.

The third point shows that the knotted and spiral configurations are not to be regarded as representing fundamentally different structures. They must represent the same structure in different configurations. We must then assume that the knotted configuration is in reality a twisted configuration (T) as a form of the spiral configuration (S). Thus the "knots" or "dots" seen in the telophase and interphase do not represent chromomeres in any sense, but the spiral turns in the spiral configuration. These conclusions seem to serve to explain certain points relating to the spiral structure which are debatable to-day. We shall consider them below.

- 1) In metaphase and anaphase, the chromosomes are generally in a highly dehydrated state, and it is highly probable that in these

chromosomes the DS or DT configuration predominates over the CS configuration. The chromonemata of the DT configuration will appear as smooth strands or strands of knotted appearance or some such, when fixed with ordinary methods. If in the chromosome two such strands or chromatids are contained (cf. KUWADA, 1939) and lie parallel with each other, a longitudinal clear space may be seen between them. This has been observed repeatedly by earlier workers and was called the anaphasic or telophasic split. The anaphasic and telophasic splits can, therefore, be understood also from the view point of the spiral structure of the chromosome and not incompatible with this theory. If the two strands (chromatids) are twisted to some extent, and present a configuration of the relational spiral, the spiral is a chromatid spiral and not a chromonema spiral. There would be in this case a great possibility of taking it erroneously for a chromonema spiral, if the chromonemata assume the twisted configuration making the chromatids appear to be much thinner than when the chromonemata are of the spiral configuration. The number of the spiral turns of the chromonemata at definite stage should practically be definite in definite chromonemata, but the number of the spiral turns or the twists between the chromatids, being relic ones of the old spiral should be much less than in the former (i.e. chromonemata) and may be variable (KUWADA, 1939). Fig. 1 of ROY (1936) may be taken as an example showing these twists, variable in number, between the two chromatids containing the chromonemata of twisted configuration. Thus, by distinguishing between the chromatid spirals or twists and the chromonema spirals, we can understand the reason why the number of twists or spiral turns can be variable in the anaphasic and telophasic chromosomes.

2) This distinction between chromatid spirals and chromonema spirals must be made also in the case of meiotic chromosomes. The so-called major spirals are the chromatid spirals and the minor spirals are the chromonema spirals (KUWADA, 1939). In the case of large chromosomes, the chromatid spirals assume the spiral configuration in metaphase and anaphase, but the chromonemata may be of a twisted configuration or some such form, and consequently, they are usually fixed in these cases as strands with a smooth or corrugated surface, and hence also no spirality is visible in them. It first becomes visible only when the chromonemata are subjected to a certain treatment by which the transformation of the twisted configuration into the spiral is caused. The minor spirals really exist, but in a dehydrated condition their spiral configuration is transformed into the twisted configuration, so that their existence becomes hardly perceptible. It should be for this

reason that the minor spirals generally appear concealed in living material and in that fixed with ordinary methods. Various chromatid configurations reported in meiosis can only be harmonized when it is assumed that the minor spirals that are visible in one configuration are concealed in another (KUWADA, 1939).

Special treatments of chromosomes can disclose their structure, but the result from fixed material may be more true to the natural configuration which they assume at definite stages. If the non-visibility of the minor spirals in fixed material is simply taken as an artifact due to fixation, this is probably erroneous. The results obtained with fixed material seem more reliable than we generally assume. It seems highly probable, that if in any stage the spirality is not visible in fixed material, the configuration taken up by the chromonemata in the living state would be in this stage some other than the spiral configuration. The chromonemata must assume various configurations in living conditions, which are derivable from their spiralized condition.

3) From the results of SINKE's hydration and dehydration experiments, especially from those obtained with the erythrocyte nucleus of *Triturus pyrrhogaster*, it seems to us that the fine structure of the nucleus is most simply explained by the assumption that the chromosome contains four chromonemata in it as believed by NEBEL (cf. KUWADA, 1939). It is, however, essential actually to determine whether this interpretation is correct or not. The determination is of course not an easy task, but the importance of the investigation of the structure in question in connection with the question of the number of chromonemata contained in one chromosome may be considered here briefly (Fig. 12). In the following considerations, it is assumed that the chromonema spiral is an orthospiral (the unbalanced spiral of ABRAHAM, 1939).

I. One chromonema is assumed. In this case the daughter chromosomes (chromatids) are separable at anaphase only when the two daughter chromonemata are coiled each independently in the spiralization to form two independent chromatids. The anaphasic chromosome is of the simplest structure containing only one single-stranded chromonema spiral in it. This corresponds to DARLINGTON's view of the chromosome structure.

II. Two chromonemata are assumed. In this case, two cases can be considered. a) The two split halves of the chromonema are coiled both together like one thread. The anaphasic chromosome contains a double-stranded chromonema spiral. b) The two daughter chromonemata are coiled each independently so as to produce two

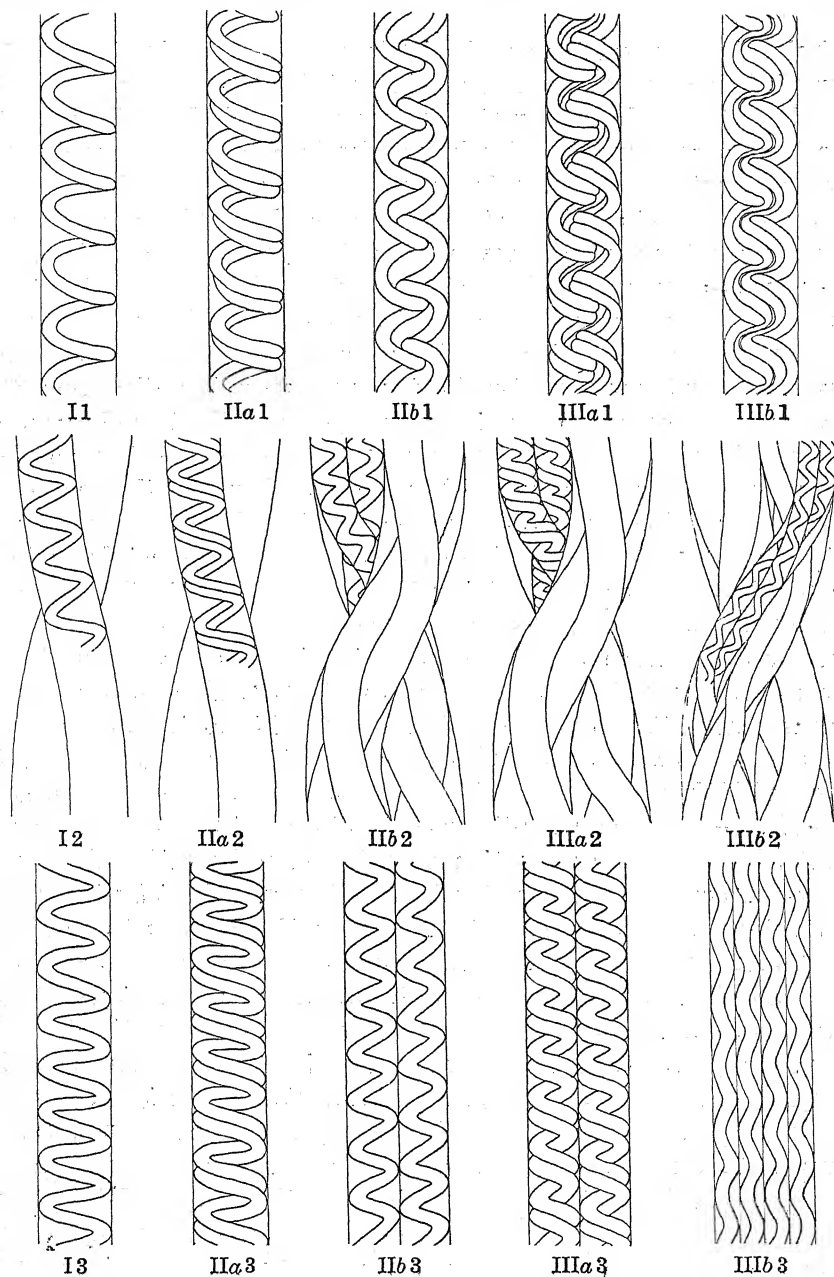


Fig. 12. Diagram showing five possible cases of chromosome cycle on the supposition that the longitudinal splitting of the chromonemata takes place in the early prophase and that the chromonema spirals are of the orthospiral type. 1, spiral stage; 2, late prophase; 3, anaphase. For IIIb 3, compare it with the diagram F of NEBEL and RUTTLE (1936). In IIIb 3, the half-chromatids are illustrated running parallel along each other in order to show each half-chromatid clearly, but the two chromonema spirals in each chromatid may be pushed into each other as in IIIb 1, so as to assume a more regular spiral configuration than shown in the diagram (IIIb 3). Further explanation in the text.

spirals. In this case the anaphasic chromosome contains two single-stranded chromonema spirals or chromatids.

III. Four chromonemata are assumed. We have here again the two cases *a* and *b*. *a*) The anaphasic chromosome consists of two chromatids each containing one double-stranded chromonema spiral. *b*) The two chromatids of the anaphasic chromosome contain two single-stranded chromonema spirals or half-chromatids each. This case corresponds to NEBEL's view of the chromosome structure.

In all these cases it is assumed for the sake of simplicity, that the old or relic spirals have been completely drawn out before anaphase, and consequently in the accompanying diagrams the anaphasic chromosomes are shown straight, and the two chromatids (II*b* and III*a*) or the four half-chromatids (III*b*) in the chromosomes are represented as lying parallel with each other. In the case III*b*, it seems probable that the drawing out of the old spirals causes the intertwining between the chromatids (the half-chromatids in III*b* 2) too, but such an intertwining is also not shown in the diagram (III*b* 3).

Now, we have strong evidence to show that the anaphasic chromosome consists of two chromatids (AISIMA, cf. KUWADA, 1939), and we have little room to uphold the possibility of the assumptions I and II*a*. It seems then sufficient to consider only the cases II*b*, III*a* and III*b*.

If case II*b* represents the real structure of the chromosome, we must abandon the explanation that the transformation from the fine structure to the coarse structures is brought about by the union of the two sister chromonemata, and reconsider the question of how the "fine" structure is caused. If, on the other hand, either case III*a* or III*b* represents the real structure, the explanation of the fine structure which we are attempting will remain without much alteration. The configuration presented by the chromonemata in this structure should, of course, be different in these two cases; in III*a* two sister chromonemata (secondary sisters of NEBEL, 1933) form a chromatid, coiling together, while in III*b* each chromonema forms a half-chromatid, coiling independently. In the latter case, the half-chromatids may also present the knotted appearance, and it may be expected that the nucleus of the fine structure with individual chromonemata of twisted configuration (FT) would exist, but in the former case it cannot, because in this case the chromonemata of twisted configuration cannot be single. So far as our observation goes, we have as yet met with no clear case where the fine structure showing twisted configuration is observed. This is certainly negative evidence, but we may provisionally exclude the



case IIIb from consideration for the moment.

The question may then rest on the point as to which case, IIb or IIIa, is the true one. In these two cases the chromosome structure is quite identical excepting that the chromonema spiral is single-stranded in the one and double-stranded in the other. Since in the dehydrated state of the matrix the double-stranded spiral would appear to be a single-stranded spiral, the distinction between these two types of spiral should only be possible when the matrix is hydrated. The investigation of the nucleus of the fine structure seems, therefore, to be very important in the solution of the question of the chromonema number in chromosomes. Some authors deny, from the results of chromosome fragmentation induced by X-ray treatment, the possibility of chromosomes each containing two or more chromonemata (DARLINGTON, 1932; GENTCHEFF and GUSTAFSSON, 1939), but such an argument does not necessarily hold. We should rather distinguish here between the chromosome division and the chromonema number. The chromosome among the components of which the matrix should be included may remain undivided irrespective of the number of chromonemata which it contains.

If the F structure really exists, and if it is to be represented by the symbol FS only, the case IIIa will remain after all one which represents the true cycle of the chromosome. It is, however, not an easy task to prove substantially the existence of the FS structure and especially that it and it alone has the appearance of the F structure. The most important point which we must bear in mind in this line of investigation is that there is a danger of mistaking an HS structure for the FS structure. The structure which we take for FS is that structure in which the chromosomes show a reticulate appearance presenting an aggregate structure of the nucleus as a whole. And yet, even we ourselves are impressed with a suspicion that we might have mistaken some HS structure for the FS structure, though a nucleus can never correspond perfectly to the type of any structure.

It is left for further investigations, to find whether the FS-structure actually exists or not, but it is an observed fact that a structure exists which appears very much finer than the structure presented by the nucleus in which the chromonema configuration is DT, DS, TS or CS. This apparent fine structure, of which the true structure remains as yet undetermined is called for the present simply fine structure without using any symbol such as FS or FT. The "fine structure" may be, therefore, a collective term covering different structures of a similar appearance, or a term indicating one of the other structures presenting a different appearance.

4) A few lines may be added here about the opinion of ABRAHAM (1939) on the type of the chromonema spiral. On the basis of his observation, the author has concluded that the chromonema spiral must be of the "balanced" type (anorthospiral). He admits that if the chromonemata divide two or three mitotic cycles prior to their separation into daughter chromosomes, "then the evidence on which the balanced spiral mechanism was shown to account for the observations in *Lilium* may not alone be sufficient to disprove the probability of the unbalanced spiral also giving rise to the appearances described". He has taken into consideration the case which corresponds to our case IIIb, but has drawn no attention to case IIIa which differs from the scheme that he has found in his investigation only in the point that the spirals which he assumes to be single-stranded are double-stranded, a point which is decidedly very difficult to determine. If in *Lilium* which he studied the chromosome cycle is such a one as we represent by the case IIIa, the chromonema spiral can be of the "unbalanced" type. The fact that the spiral configuration and the twisted configuration of the chromonemata are mutually transformable is, on the other hand, rather difficult to explain if the chromonema spirals are "balanced" spirals. If they are such spirals, the compensating internal twists will cancel the external twists, when they take the "twisted" (drawn out) configuration, and new spiralization must take place in each transformation into the spiral configuration. But, it seems highly probable that the spiralization takes place only at one definite stage in the chromosome cycle where it occurs under a certain complex condition which is peculiar to that stage.

5) The fact that in most staminate hair nuclei the twisted or the TS configuration predominates, clearly explains the reason why the elongated nuclei show the negative optical sign in respect to their long axis. According to BECKER and KOZBIAL (1937, cf. BECKER, 1938), the optical sign of fixed chromosomes (in mitosis) is negative, when they are thin and long, and is positive, when thicker and shorter. These results are in accord with our view that the optical sign is determined by the configuration of the chromonemata. When the chromonemata are of the twisted configuration and the chromatids containing them run parallel or are twisted on each other only loosely or slightly, the chromosome will show the negative sign, and if in these chromosomes the twisted chromatids assume the form of spiral, if hence the chromosomes are of the double-coiled structure, the optical sign will be positive. The chromosomes of the former case may be roughly said to be of the single-coiled structure. If in these two cases, single-coiled and double-coiled, the chromo-

nemata assume the spiral configuration, the optical sign will be reversed in both cases; positive in the case of the single-coiled structure and negative in the double-coiled structure. BECKER and KOZBIAL are convinced of the fact that somatic chromosomes are negative when they are thin and long, and positive when thicker and shorter. According to the authors, in the former case the chromosomes "do not show any distinct microscopic structure", while in the latter they "show a spiral structure according to the scheme of NEBEL, GEITLER and others". If, then, still shorter and thicker chromosomes are optically negative, how should we interpret the reason why they are not positive. The simplest explanation is this: Because in these chromosomes the chromonemata which are of the same configuration as those of the chromosomes of positive sign are coiled into major spirals. If in these chromosomes major spirals are microscopically observed, it may be regarded as being demonstrated by the polarisation microscope that what we have called the minor spirals are actually of the spiral configuration. If in these chromosomes the optical sign is positive, this sign must show that the minor spirals assume a twisted configuration in this case. The investigation with the polarisation microscope can, therefore, not only confirm the microscopical results by ordinary light, but also give some information on the finer structure which the ordinary microscope can not reveal with accuracy. We wish here to emphasize, however, that microscopically visible structures must be taken first into account in the discussion of a morphological problem, though the basic finer structures which are not directly visible are also equally important.

In concluding, we may say that in living condition the spiralized chromonemata may not always present the spiral configuration, but may also be of the twisted configuration. These configurations are mutually transformable, and from this fact it seems likely that the chromonema spirals are orthospirals rather than anorthospirals. It seems also probable that the reason why the new coils are visible in the spiral stage only through a certain special treatment rests on the ground that in the first process of spiralization no regular spiral is formed. A twisted configuration in the beginning of spiralization seems to develop later into the spiral configuration, a development which may result in the shortening and thickening of the chromosomes. Both in living and fixed materials, the spirality is visible only when the chromonemata present the spiral configuration, and not when they assume the twisted configuration. Special treatment can, on the other hand, transform the twisted configuration into the spiral configuration, and thus it can reveal the true

structure which may appear in different configurations. Strictly speaking, the result of the special treatment may be an artifact, but it is the artifact characteristic of the structure. One may call it an artifact, indeed, if he refers only to the particular stage or condition at which the structure appears otherwise, but one cannot call it such in a general sense, because the structure can appear physiologically in other stages or conditions in the same form as it can artificially as a result of a special treatment.

### Summary

1. Spiralized chromonemata present different configurations. The configurations can be classified into two general types, the spiral configuration and the twisted configuration.

2. These configurations are mutually transformable from one to the other. These changes are experimentally demonstrated in the resting nucleus.

3. In the twisted configuration, the nucleus shows a diffuse structure, while in the spiral configuration it presents an aggregate structure, the chromosome territories being perceptible.

4. When staminate hairs are observed in water medium, the prophasic nucleus is transformed directly into the resting nucleus without passing metaphase and anaphase, an "abbreviated mitosis" which is comparable with the endomitosis. It is pointed out that in one mitosis the chromosome splitting never takes place twice, but that it only appears to do so, when one of the two successive mitoses is an "abbreviated" one.

5. From the fact of the spiralized chromonemata presenting different configurations, the problems of 1) the anaphasic or telophasic split, 2) the minor spirals, 3) the fine structure of the nucleus in connection with the problem of the chromonema number in chromosomes, 4) the type of the chromonema spirals, and 5) the optical sign of chromosomes are discussed.

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## Two Problems in the Interpretation of Meiosis in Plants

By

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Chromosome structure in the pollen mother cells of Liliaceous plants were being studied in the course of an investigation into the structure and behaviour of plant chromosomes. In the P.M.C. of *Tulipa* and in that of *Rhoeo* certain features were noticed which offered problems in interpretation. The meiotic chromosomes of both these plants have been studied before (Newton (2), Darlington (10), Nebel (11) ) and there is a good deal of corroboration that the facts of observation are correct. It is here attempted to explain the problems and the nature of observations from which they arise.

### Material and methods

Smear preparations of pollen mother cells were used exclusively. The plants studied were the cultivated garden tulips all included in the species *Tulipa Gesneriana* and the exotic garden plant *Rhoeo discolor* (Hance). A certain amount of parallel studies were made in the P.M.C. of English Snow drop, *Galanthus nivalis*. In *Tulipa* and *Galanthus* the meiotic divisions are completed in the bulb stage and therefore, all the materials were obtained as bulbs. The tulips included the diploid varieties, *Rawenhof*, *Clara Butt* and *Rubra maxima* and triploid variety, *Inglescombe yellow*. Some of the *Galanthus* bulbs were diploid and some were tetraploid. The smears were fixed in a 2½ per cent solution of Merck's sodium uranium acetate and the details of the technique are described elsewhere (15). The smears fixed in this solution gave good results with crystalviolet as the stain, stained by Newton's iodine-violet method. Osmic-chromacetic fixative was tried as a control. It was found that the new fixative used was as reliable as the more commonly used one.

### Observations

In the course of study it was found that the pressure exerted during the process of smearing gave rise to artifacts. The following expedient gave preparations which were used as a control in the interpretation of smears. When the tips of the long anthers of

*Tulipa* are excised a drop of fluid exudes from each anther, containing a large number of P.M.C. which have been extruded by the normal and natural pressure within. When these drops were transferred to a slide and fixed the cells showed undisturbed outlines as well as a general even staining property.

In *Tulipa* the commencement of division was as follows:

Anthers from bulbs bought in the beginning of September were not in a smearable condition, the P.M.C. being held together as a compact tissue. This condition persists in some varieties for a few weeks and it is therefore, evident that the resting stage after the last premeiotic division intervenes for a considerable time. Even after the P.M.C. become loosened and are, therefore, in a smearable condition there ensues a stage lasting for one or two days when the nuclei are fixed badly, being either structureless or coarsely granular. After this stage the P.M.C. show good fixation and different stages of division occur freely. But most of the stage are from zygotene or pachytene onwards and there are few cells in which a leptotene stage can be identified with definiteness. It is, therefore, inferred that the loosening of the P.M.C. indicates a commencement of division and that the initial failures are in the leptotene stage.

The chromosomes in the earliest stages are in the form of thin long threads, so long as to form a tangled mass in the periphery of the nucleus. The interior of the nucleus is relatively empty of chromosomes. The 24 long threads are seemingly disposed in an irregular manner this apparent confusion is considered to be of theoretical importance. In the cells interpreted as being in the leptotene stage there is no indication of pairing, the characteristic of twin parallel threads being absent. Also the chromosomes are of uniform thickness. The zygotene stage is characterised by the presence of chromosomes in two grades of thickness. The contrast in size is brought about by the close union of pairing chromosomes in some sections of their length. These paired sections are markedly thicker than each of the homologue which contributes to them. By pachytene the pairing is complete. In diploids the fixation of pachytene chromosomes is such that the presence of two homologues in each double thread is obscured. At pachytene the threads are thicker than at leptotene and apparently shorter, for their course can be more easily traced. By diplotene further shortening takes place and the bivalents are spatially free of each other.

In triploid *Tulipa* the leptotene and zygotene nuclei are crowded by the 36 long chromosomes. The assortment of partners is in threes. A distinct pachytene was not observed.

In diploid and tetraploid *Galanthus* pachytene is the earliest identified stage. The 24 and 48 long chromosomes form a tangle in the periphery of the nucleus. In the tetraploid no trivalents or tetravalents are formed. The diplotene, diakinesis and metaphase are very much like that of diploid *Tulipa*.

To recapitulate, in all the above type, chromosomes are long and numerous. The prophase commences when the nuclei are crowded by the thin long chromosomes. In all the types the homologues swing together in twos or threes smoothly and unfailingly. The regularity is verified by the complete absence of interlocking bivalents at diplotene and metaphase. There is nothing in these observations which contradicts the observation of Newton (11) and Newton and Darlington (12) on *Tulipa* and is in general agreement with the observation of other workers in long chromosomed plants.

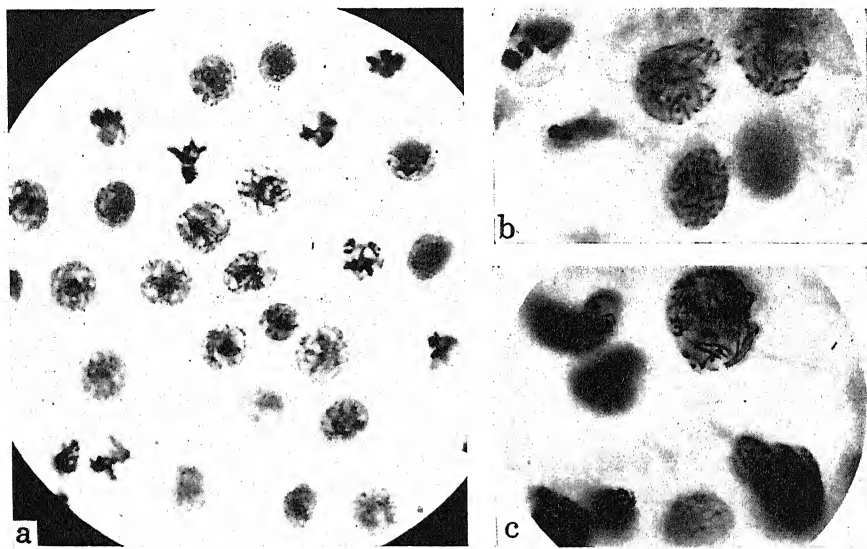


Fig. 1a, 1b. Pachytene stage of pollen mother cells in diploid *Tulipa*. 1c. Zygotene stage of pollen mother cell in triploid *Tulipa*.

It is intended, however, to emphasise certain physical aspects of division. The prophase nucleus can be visualised as a ball of thread which is more or less hollow in the centre, all the chromosomes traversing the periphery. Also no apparent order of arrangement can be detected in the two genera. Often it is not possible to trace a chromosome from beginning to end. The photomicrographs 1A, 1B and 1C are of zygotene and pachytene stages in *Tulipa* and are included to show the type of preparations from which the observations were made. Fig. 1C of zygotene in triploid



*Tulipa* shows to a certain extent the manner of distribution of chromosomes. These facts are produced to point to the problem: how a smooth regular pairing of chromosomes is achieved when the homologues are disposed in an inextricable confusion.

In *Rhoeo* the earliest stage fixed adequately is the metaphase of the first division. In this stage the chromosomes seem undivided. The clone used was a thirteen chromosomed type in this species. In a large number of cells all the thirteen chromosomes are arranged in a circular ring. In other instances the ring is broken forming one, two or three chains. In all cases the attachment between chromosome and chromosome is strictly terminal. No instance of complete side by side pairing has been found. No structure resembling a chiasma has been found. Also in a large number of cells the chromosomes showed internal structure, probably spiral structure.

To put it in a clearer form, the first metaphase of *Rhoeo* closely resembles that of the thoroughly studied *Tradescantia* (1, 2, 6, 9, 13). Each metaphase chromosome is probably composed of two closely fitting spiral coils. The coils of the two halves of each chromosome fit into each other so closely that it appears like a single beaded rod. The structure given here is conjectural. But it is the only likely interpretation which will fit in with the following observations:

(1) Chromosomes do not show a linear split, or a semblance of linear split. (2) The technique is good enough to show structure in the chromosomes. (3) The structure looks spiral and is probably not an artifact. (4) If the two halves of a chromosome were spatially free of each other, two distinct spirals should be seen at least in a few cells; such cells have not been found. (5) At the commencement of anaphase (namely when the different chromosomes become free of each other) a double spiral can be seen in a few cells. (6) When the chromosomes reach the poles, the sister-chromatids are distinctly spatially free of each other and reveal their previous spiral structure. These observations are adduced as evidence that the technique is not so defective as to cause two close lying threads to fuse with each other.

There are two features in the observations which are important for subsequent discussion. The first is that the chromosome pairing is terminal. Secondly, each chromosome seems undivided, thereby implying a particular form of internal structure. The photomicrographs 2a and 2b are those of first division metaphase in *Rhoeo* and are from different cells at varying magnifications. The figures show the type of preparation from which the observations are made. In some instances, both end to end pairing and the undivided beaded appearance of chromosomes are shown. There is clear evidence for

the statements, in the photomicrographs published by Nebel (10). In his figures even the spiral structure is distinct.

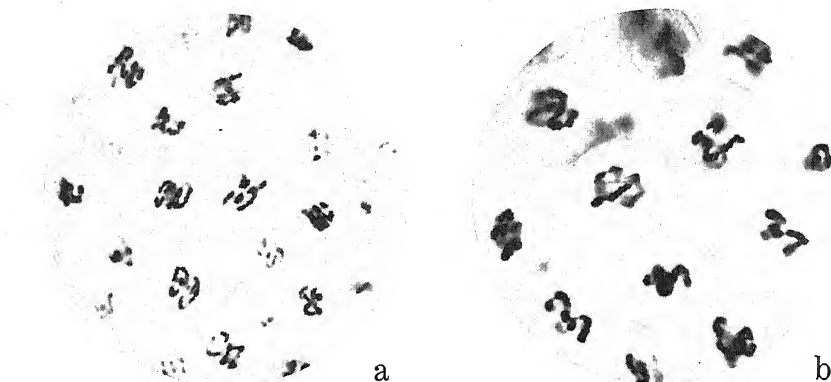


Fig. 2a, 2b. Metaphase of first meiotic division in *Rhoeo discolor*.

In *Rhoeo* the problem which arises from the above observations, is as to how the end to end attachment arose. That it is a problem is realised by trying to clearly visualise the hypothetical steps by which it arose. The details can be better dealt within the ensuing discussion.

### Discussion

The two problems can be treated together for, they seem to have a common solution. They arise only if the following assumptions are correct. (A) The facts of observation must be correct. (B) The chromosomes must have properties of a solid and not a fluid and must retain their integrity throughout division. The assumption (B) is never likely to be questioned by cytologists and may be taken for granted. There is a good deal of corroborative evidence for the facts:

(i) The first problem viz., the origin of side by side pairing in *Tulipa* can be taken first. Newton and Darlington (11, 12, 4) have studied the nucleus of *Tulipa* and their description, figures and photographs agree with the present observation. Similarly, crowded nuclei have been observed by the authors in *Tradescantia virginica* and *Hyacinthus orientalis*. Similar problem should exist in many large chromosomed plants e.g., *Fritillaria*, *Lilium*, *Trillium* and *Gasteria*.

(ii) In order to make clearer the nature of the problem the following pictorial aid may be sought (3a, 3b). In fig. 3a, which is diagrammatic, the prophase nucleus is represented by two thin threads. Two hypothetical homologues are drawn in Indian Ink,

one smooth and one granular. These two follow an independent course in the nucleus. The problem is realised by visualising a mechanism by which the two homologues can be brought together without interference from other chromosomes. In fig. 3b the homologues are represented as following parallel courses in the nucleus. Obviously the pairing would be very much simplified in this case. The presentation of problem suggests the obvious solution that the homologues may follow parallel courses in the nucleus and *are not distributed at random*. For no amount of "affinities" will remove mechanical obstacles. That is, the problem reduces itself to this: by what mechanism can regular arrangement of homologues be brought about in resting stage. It is suggested that in the premeiotic telophase, the homologous chromosomes lay close together

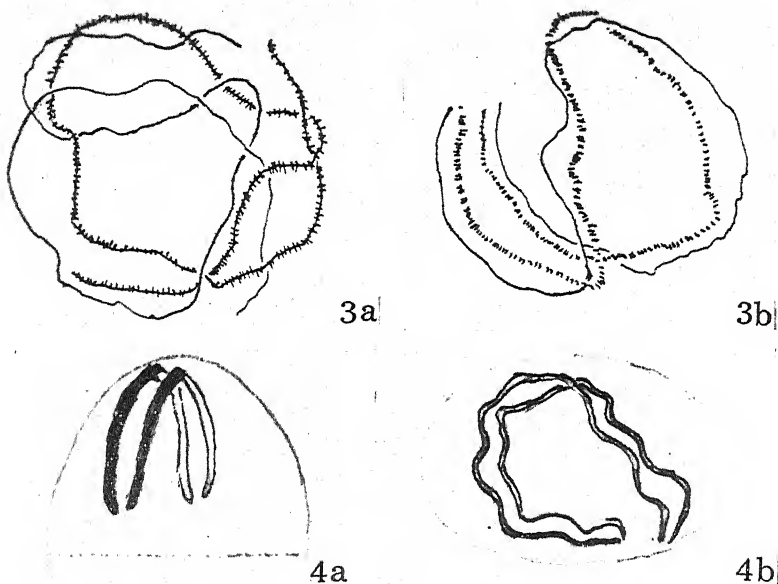


Fig. 3a, 3b. Diagrams of two possible types of distribution of two homologues at prophase of meiosis. Fig. 4a, 4b. Diagrams of a possible type of arrangement of two homologues at premeiotic telophase.

and expanded closely together into the resting stage. Figs. 4a and 4b illustrate the point. The telophase is suggested because at that stage the chromosomes are short and mobile. Some such mechanism will result in homologues lying in proximity throughout the resting stage.

In *Rhoeo* also it is highly probable that the facts of observation are correct. That there is spiral structure in the chromosome will not be contested. That the pairing of chromosomes is end to end

is also well established (Darlington (2), Nebel (10), and Sands (13)). That the two halves of a chromosome are close together with their coils interlocking is shown in the present study and in Nebel's photograph. In the closely allied genus *Tradescantia* which has been studied thoroughly (Darlington (2), Sax (7), Kuwada (8), Nebel (10) and La Cour (9)) a similar condition exists. This evidence is adduced to show that the seemingly undivided condition of metaphase chromosomes may represent the natural appearance and not an artifact.

(iii) To further illustrate the problem the photograph of a wire model is included (Fig. 5). The model represents a pair of chromo-



Fig. 5. Wire model of a hypothetical chiasma between two chromosomes.

somes having an interstitial chiasma. There are four wire coils in all representing four chromatids and the crossover is between two chromatids alone. Such a model will show that if there is to be a terminalisation of chiasma, there will be mechanical obstructions. Chiasma movement will be possible only if the two halves are spatially free of each other. This model is brought in to show that the attachment between chromosomes in *Rhoeo* are not likely to be due to interstitial chiasmata which have terminalised. The point arises because a somewhat similar explanation has been offered by

Koller (6). If alternative explanation is sought one such is as follows: The terminal attachments may have no special genetic significance at all and may not be due to crossover at all. Such a connection may be a survival from the premeiotic telophase where chromosomes expanded into resting stage with their adjacent tips touching. This explanation is very similar to that given for the previous problem. Possibly other solutions as suggested by Darlington (3) may exist. The present interpretation has the advantage that it can be verified or disproved by purely cytological methods.

The problems are taken up as they have a great bearing on theories of meiosis. If technical difficulties are overcome and premeiotic telophase thoroughly studied in any large chromosomed plant the first solution can be checked. If it is found correct, it will be highly pertinent to the 'precocity theory of meiosis' offered by Darlington (4).

### Summary

Two problems in interpretation of meiosis were noticed in a study of meiotic chromosomes of *Tulipa* and *Rhoeo*.

The first is as follows: The prophase nuclei of *Tulipa* is a

dense tangle of threads. Invariably and smoothly the tangle resolves itself into bivalents and trivalents by metaphase. The problem arises, how is such a pairing mechanically possible?

The second problem is as follows: The metaphase chromosomes of *Rhoeo* are linked to each other end to end. Each chromosome is composed of two closely fitting spiral chromatids. It is improbable that the terminal attachments are due to terminalised chiasmata. An alternative solution is wanted.

The problem arises only if (i) the facts are correct (ii) if the chromosomes retain their identity throughout the division.

(i) is corroborated by other workers' publications and (ii) is an incontrovertible assumption.

It is suggested that both the problems have a common solution. Possibly a certain arrangement of chromosomes in the premeiotic telophase facilitates the prophase pairing in *Tulipa* and originate metaphase figures in *Rhoeo*.

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## Striation Pattern of the Epithelium-Cells of Yolk-Sac Membrane and Embryonic Body of *Oryzias latipes*

By

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In developing embryos of *Oryzias latipes*, the author found, in the summer of 1928, an interesting striation pattern, made of delicate cytoplasmic striations, in the cytosome of large, polygonal epithelium-cells of the yolk-sac membrane (Figs. 1, 6). The same pattern is also found in the epithelium-cells of embryonic body and of young fish, especially of its fins.

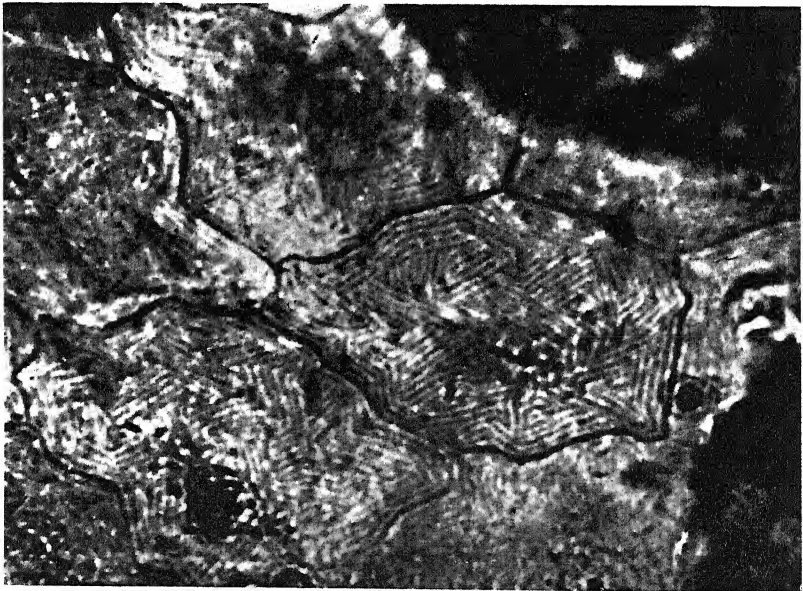


Fig. 1. A photomicrograph of the ectodermal cells of the yolk-sac membrane of the third day embryo showing the striation pattern. The photograph is taken from the preparations of the yolk-sac membrane fixed in Stockard's solution, but not stained. Note sharp contour of the ectodermal cells and their striation pattern. Dark cells are blood cells and a large dark patch on the right-upper hand is a blood vessel.  $\times 630$ .

Fortunately, the striation pattern can be microphotographed from the preparations of the yolk-sac membrane that are fixed with Stockard's formaline-glycerin mixture (Fig. 1).

Since no such striation pattern in cells has hitherto been reported, several characteristic features of the pattern and its genesis will be noted in the present paper.

The eggs used for the present studies were mostly those of red strain (genotype;  $bbRR$  or  $bbRr$ ), as in these embryos, it is easy to observe the striation pattern in the cells owing to the scarcity of melanin granules in the melanophores distributed underneath. In other strains, observations are more difficult, but the striation pattern is quite identical.

The striation pattern of the epithelium-cells of the yolk-sac membrane can be observed in living embryos by narrowing the iris-diaphragm or by making the optical axis oblique of the microscope. In embryos fixed with Stockard's solution, the striations can also be seen on the transparent yolk-sac. For detailed observation of the pattern, the fixed material is preferable, because, after the removal of the egg-chorion, the superficial layer of epithelium-cells can be peeled off from the underlying layer of periblast of the yolk-sac membrane. Then the isolated pieces are mounted in glycerin or in water unstained. Suitable magnification for observation of the striation pattern is about 400.

### Cytological features of the striation pattern

Figure 1 is a photomicrograph of the striation pattern of the epithelium-cells on yolk-sac membrane. It was taken from a preparation of the isolated pieces of the membrane fixed with Stockard's solution. The magnification is about 630.

The epithelium-cells of yolk-sac membrane are large and polygonal in shape, and their contour is well defined and thick (Figs. 1, 5, 6). A large oval nucleolus, (rarely two), is found in the nucleus containing scattered chromatin granules. In the cytosome, beside minute cytoplasmic particles distributed uniformly, there is no other constituent than the striations.

The peripheral striations of the pattern are generally arranged parallel to the cell border, and not parallel to the contour of nucleus. The pattern, however, is made up of many groups of parallel striations that are arranged in various directions and it looks like a mosaic floor (Figs. 1, 6). The parallel striations are about one micron apart from one another.

It should be noted that in these cells, the nucleus lies on a different level from that of the striation pattern. Attempts to discern another set of the pattern on the under surface of the epithelium-cells so far failed.

Examined with high magnification, it was made clear that each striation is made up of minute particles of cytoplasm closely arranged in a line (Figs. 1, 5, 6).

Whether this pattern is within the cytoplasm or whether it is on the cell surface is a difficult point to settle. The fact that these lines are made of particles suggests the striations are protoplasmic bodies, but another fact that the striations are unstainable with usual cytoplasmic stains such as eosin and acid fuchsin indicates that they are metaplastic in nature or even of a membranous character.

During the rhythmic contraction of the yolk-sphere of this fish (see, Yamamoto, '31) the contour of epithelium-cells becomes crumpled, and the striations are like-wise distorted. But after the wave of contraction passes, the cell contour goes back to the original shape and so does the striation pattern.

*Developmental features of the striation pattern.*

The ectoderm cells of the blastoderm are somewhat polygonal in shape in the early blastula stage and they are called the epidermic stratum of the blastula-blastoderm. The size of these cells is still very small at this stage (Fig. 2A). They grow larger as the development of the blastoderm proceeds until the germ-ring has travelled further down to cover the whole yolk-sac, leaving a small yolk-plug. At this time the cells of the epidermic stratum have become the cells of the ectoderm of the yolk-sac membrane and of the embryonic body.

About eleven hours after fertilization (about 4.30 pm. of the first day, room-temp. 30°C), the germ-ring comes to cover about 2/3 of the yolk-sphere leaving a group of oil-spheres, and there appears a triangular embryonic shield containing the embryo (Fig. 3). At this stage the polygonal ectoderm cells with well-defined cell contour are established in the area of embryonic shield and in the germ-ring near it (Fig. 3C), while the ectoderm cells on the opposite side from the embryonic shield are irregular in shape and their cell membranes are only partly formed (Fig. 3A). At this stage the ectoderm cells contain cytoplasmic particles distributed uniformly and a large nucleus, in which a single nucleolus, (rarely two), is found.

In the ectoderm cells near the embryonic body that has attained to the 13-somite stage (Fig. 5), when the pulsation of the embryonic heart is about to commence about twenty-one hours after fertilization (about 4.00 pm. of the second day, room-temp. 30°C), from uniformly distributed particles (Fig. 4) there appear incomplete striations made of closely arranged cytoplasmic particles (Fig. 5). These pieces of striations are the first indication of the striation pattern, which is completed on the third day of development (Fig. 6).



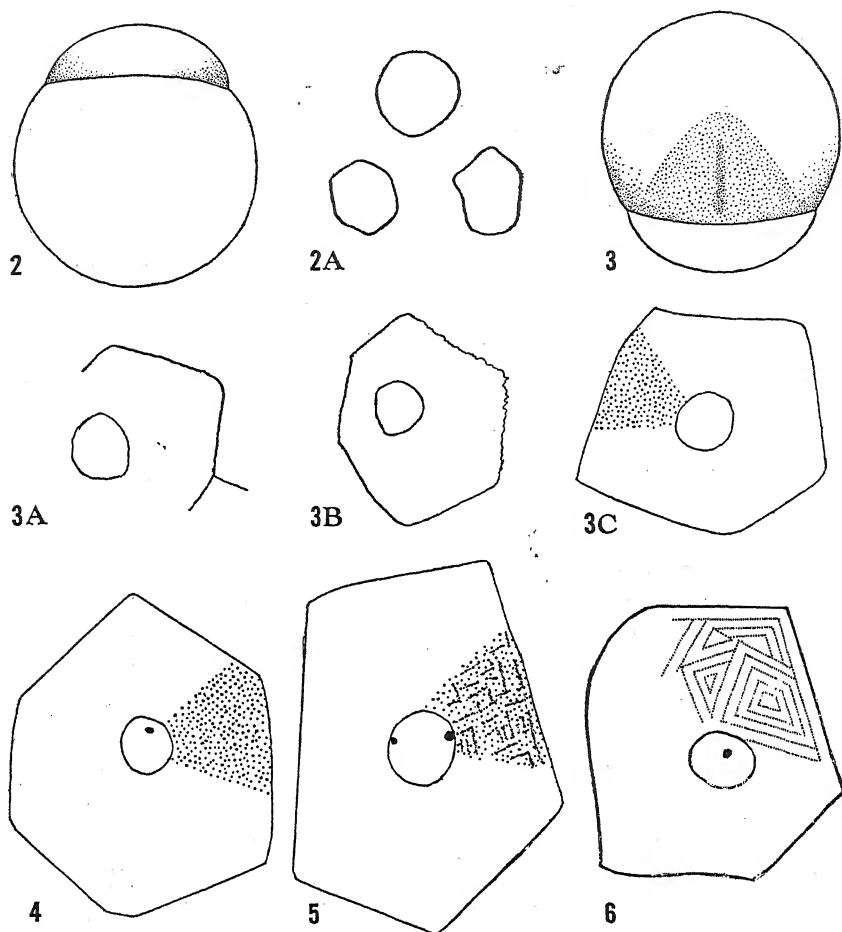


Fig. 2. An embryo covered with the blastula-blastoderm about three hours and a half after fertilization. (room temperature about  $30^{\circ}\text{C}$ ).  $\times 28$ . Fig. 2A. Surface view of the cells of the blastula-blastoderm at the stage of Fig. 2.  $\times 466$ . Fig. 3. An embryo in which the germ-ring has reached to two thirds of the yolk-sphere, and the triangular embryonic shield has been formed. This stage is attained at about nine hours and a half after fertilization. (room temperature about  $30^{\circ}\text{C}$ ). Fig. 3A. An ectodermal cell found on the opposite side from the embryonic shield at the stage of Fig. 3. The cell membrane is partly formed.  $\times 466$ . B. An ectodermal cell found on the germ-ring far apart from the embryonic shield at the stage of Fig. 3.  $\times 466$ . C. An ectodermal cell found on or near the embryonic shield at the stage of Fig. 3. The cytoplasmic particles are seen.  $\times 466$ . Fig. 4. A polygonal ectodermal cell found on the yolk-sac membrane of the 4-somite embryo. The cytoplasmic particles are uniformly distributed.  $\times 466$ . Fig. 5. An ectodermal cell of the yolk-sac membrane near the head of the 13-somite embryo. The first indications of cytoplasmic striations are seen.  $\times 466$ . Fig. 6. A polygonal ectodermal cell of the yolk-sac membrane of the third day embryo. The striation pattern has completely been established.  $\times 466$ .

No correlation between the rythmic contraction of the embryo and this striation pattern can be found, for when the former begins (about twelve hours after fertilization, room-temp. 25°C-27°C) the striation pattern has not yet been formed, and the latter persists even when the contraction becomes less and less prominent after the fourth day of development.

Drs. K and J. C. Dan, of the Misaki Marine Biological Station, were kind enough to read through the manuscript.

### Summary

1. Remarkable cytoplasmic striation pattern is found in the epithelium-cells of yolk-sac membrane and of embryonic body of *Oryzias latipes*, especially of its fins.

2. The striation pattern is composed of several sets of parallel striations that are arranged in mosaic to every directions. The peripheral one or two striations are, however, arranged parallel to the border of cell, while there is none of the striation that is arranged parallel to the border of nucleus.

The parallel striations are about one micron apart from one another.

3. Each striation is made up of minute cytoplasmic particles closely arranged in a line.

4. The striations, however, are unstainable with usual cytoplasmic stains such as eosin and acid fuchsin.

5. The first indication of the striations appears in the epithelium-cells of yolk-sac membrane of 13-somite embryos. At this stage the minute cytoplasmic particles, which hithertofore distributed uniformly in the cytosome, come to be arranged closely in a line, and then the pieces of striation thus formed become continuous and finally establish the striation pattern. The complete striation pattern is found in the epithelium-cells of yolk-sac membrane of the third day embryos.

6. No correlation between the striation pattern and the rythmic contraction of yolk-sphere was found.

## Basikaryotype and Its Analysis<sup>1)</sup>

By

Y. Sinotô and D. Satô

(With one figure)

Received March 13, 1940

### Introduction

Chromosome studies have been actively carried on in the field of cytogenetics since Rosenberg (1904) observed the chromosome behavior in the meiosis of a hybrid between two *Drosera* species, and the multiple relation of the chromosome numbers or polyploidy has been found in allied forms.

Generally two sets of chromosomes are found in somatic cells, while only one set is found in the sexual cells or gametes, the former being called diploid ( $2n$ ) and the latter haploid ( $n$ ). The polyploids which have more than two sets of chromosomes are called triploid, tetraploid, pentaploid, etc. according to the number of chromosome sets observed. In these cases the chromosome numbers in the somatic cells are expressed  $3n$ ,  $4n$ ,  $5n$  etc. when the basic number is symbolized as  $n$ . The use of these formulae to represent the gametic numbers of chromosomes is not adequate. The symbol  $x$  was suggested by Sansome and Philp (1932) for the basic number instead of  $n$ , while on the other hand the senior author (Sinotô 1929) proposed  $b$  which is the initial letter of a basis or a basic number of chromosomes. This symbol has been used in our laboratory since then and has been supported by Gates (1935) and his school. Gates emphasized that it was necessary to find a new symbol in place of  $x$  in order to avoid confusion, since  $x$  would then be employed in three ways, namely in the case of X-rays, X-chromosome and as the symbol of the chromosome number.

Thus the investigation on the chromosome numbers has lead to the development of the conception of the basic number and recent studies have resulted in the elucidation not only of the chromosome numbers but also of the size and shape of the chromosomes. Delaunay (1922) observed the following fact in some genera; namely that all the species of a certain genus studied has generally a similar chromosome type especially in size classes. He gave a term "Karyotypus" to this particular chromosome type in the genus

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1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 244.

and thought that it corresponds with the systematic unit, genus. Levitsky (1924) independently suggested a new conception of "Karyotyp" which was different from that of Delaunay in that no assumption was made concerning its relationship to a systematic unit such as the genus, the meaning he ascribed to it being "the type of chromosomal complex characteristic of any individual or group of allied forms" (Sharp, 1934, p. 126). The karyotype<sup>1)</sup> has become important in chromosome studies and progress made in the method of karyotype analysis was advanced having stimulated the investigators who came to take an interest in the study of the morphology of chromosomes in a set corresponding to the basic number. This has necessitated suggesting a new conception of karyotype for which we propose the term "basikaryotype". In the following we will explain in detail the conception of the basikaryotype and the method of its analysis.

### Karyotype analysis

In karyotype analysis not only the length and breadth of individual chromosomes but also other morphological characteristics such as primary and secondary constrictions and trabants have to be investigated as carefully as possible. When all of the chromosomes in the set cannot actually be analyzed, certain particular chromosomes such as SAT- and nucleolar chromosomes, allosomes, sex-chromosomes and sometimes larger chromosomes need to be analyzed. Even when none of the chromosomes can be distinguished owing to their similar, often small size and shape, that is, when they are not suited for karyotype analysis except as regards their number, then basikaryotype analysis in the sense later specified may be rendered to some extent possible by adopting the conception of secondary association in meiosis (cf. appendix). For the present it is interesting to note that such secondary association of chromosomes has been reported in a number of plants most of which have small similar chromosomes.

By karyotype analysis the basic number of chromosomes and their morphology and further basikaryotypes can be determined. Such basikaryotypes, however, may be distinguished in some cases but not in others only by their morphological differences. Accord-

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1) The definition of the karyotype seems to differ with different authors. For instance, Bruun (1932) states: "By karyotype is, therefore, here meant the sum total of those nuclei which have most character in common." (p. 196). Variants in a karyotype were called "facies" and a higher rank was called "karyoforms" by Bruun (p. 196-7). We use the term karyotype in the broader sense as including the three terms used by Bruun.

ingly the question of homology of the basikaryotypes or that of the chromosomes composing the basikaryotypes must be left for further investigation, according to the method of basikaryotype analysis which will be explained later in detail. Even when the differences among basikaryotypes can not be made out by karyotype analysis in the somatic cells, these differences may be detected by observing chromosome alterations such as inversion, translocation etc. shown by the chromosome behavior in meiosis of the plants or hybrids which have the basikaryotypes in question. It is nothing but a basikaryotype analysis to look in such a manner for the homology among individual chromosomes belonging to the different basikaryotypes.

### Genome analysis

As already mentioned above, the conception of the basic number of chromosomes has been established with the progress of the chromosome studies. Winkler (1920) proposed a term "Genom" for the particular chromosome set.<sup>1)</sup> The basic number simply implies the chromosome number, but the genome suggests a genetical constitution of the chromosome group composing the basic number. A certain plant has AA genomes, and another BB, so their hybrid may have AB genomes. The homology between the genomes is induced by the mode of conjugation of the chromosomes observed in meiosis of the hybrid. Only bivalents are formed in meiosis of the plant with either AA or BB genomes. The A genome is different from or non-homologous with the B, so only univalents are observed in meiosis of the plant having the AB genomes. When the homology between the two genomes is not complete, some of the chromosomes may not conjugate but remain as univalents. In this case these two genomes are said to be "partially homologous." Thus difference or homology of genomes can be traced by using such affinities existing more or less among different genomes. This method, genome analysis, has already done much for the advancement of cytogenetics. In this line the genome analysis in *Triticum* and its allied genera by Prof. Kihara and his school is renowned.

In practice, in genome analysis, the analysators are crossed with an unknown plant to be analysed and the homology of the genomes is judged by the chromosome conjugations in meiosis of these hybrids. Thus a plant with AA genomes is crossed with a plant with unknown genomes XX to form a hybrid with AX genomes.

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1) The conception of the genome seems to have wide implication as seen by Kihara's (1939) historical and critical review on this term.

When only bivalents are found in meiosis of the hybrid the X genome is assumed to be homologous with the A and is determined as equivalent with the A genome. On the other hand only univalents may be found when the A genome is non-homologous with the X, which is then presumed as another genome B or etc. If both bivalents and univalents are formed, the A genome is partially homologous with the X which is then determined as an A' genome.<sup>1)</sup>

In recent investigations partially homologous genomes have come to be found rather frequently and moreover the existence of partial homology has been established by observation not only in the genome as mentioned above, but also in individual chromosomes themselves composing the genome. In other words the chromosome alterations such as inversion, translocation, deletion etc. have been detected in certain regions of the chromosomes. Consequently the conception of genome may be modified by taking the karyotype into consideration or it may be related to the conception of further development from the stand-point of the karyotype alteration. The conception of genome however seems to have considerable implication in so far as the papers hitherto published go and suggests further development in various lines of genetics and evolution. We shall however attempt here to deal only with the one aspect of the nature of the genome in which the karyotype is chiefly concerned.

### **Basikaryotype analysis**

By karyotype analysis made in individuals or allied groups (species, section, genus, family etc.) the basic numbers of chromosomes (bases) are determined and consequently various basikaryotypes can also be detected in them in different combinations. The resemblance of basikaryotypes is assumed to suggest a phylogentic relationship, but further investigations in regard to the homology or phylogeny of basikaryotypes make it necessary to consider hybridization between the two forms having the basikaryotypes in question. Accordingly when hybrids cannot be got by crossing them, further analysis of the basikaryotypes is impossible. On the other hand when the two plants can be hybridized, the analysis can be continued, homology between the basikaryotypes being judged from the chromosome behavior in meiosis of the hybrid.

In short, the basikaryotype can be determined by the karyotype analysis and the homology, either complete or partial, among individual chromosomes composing the basikaryotype can be deduced

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1) For detail of genome analysis, see Kihara, Yamamoto and Hosono 1936 "Studies on the Chromosome Numbers in Plants," pp. 87-98.

from the state of pairing in meiosis, which is assumed to show homology of chromosomes or parts of the chromosomes. And the latter method of investigation is what we propose to call a basikaryotype analysis. Even the mere basikaryotype may suggest that chromosome changes such as fragmentation, fusion, translocation, inversion or deletion have occurred, and in some cases it may be true, but strictly speaking this is but a presumption and it can hardly be said that any convincing karyogenetical evidence has been obtained, until the basikaryotype analysis has been made.

The basikaryotypes can be determined in the forms in which the karyotypes have been analysed in detail; for instance the karyotype in *Paeonia* ( $2n = 10$ ) (cf. Sinôtô 1938) is generally shown as follows,<sup>1)</sup>  $K = 2L_1^+ + 2L_2^+ + 2M + 2S_1^+ + 2S_2^+$ , hence the basikaryotype B is  $L_1^+L_2^+MS_1^+S_2^+$ .

In *Tricyrtis formosana* var. *stolonifera* ( $2n = 25$ ) (cf. Satô 1939),  $K = 2L_1 + 2L_2^+ + M + 2S^+ + 18S$ ; hence two basikaryotypes with different chromosome numbers are shown as follows,  $B_1 = 13 = L_1L_2^+S^+10S$  and  $B_2 = 12 = L_1L_2^+MS^+8S$ . In this case the M-chromosome suggests by its three constrictions that it has been derived from the fusion of the two small chromosomes (S).

When various karyotypes are found in one and the same species such as *Scilla permixta* ( $2n = 14, 15, 16$ ) (cf. Satô 1936), the basikaryotypes are also complicated. The I-type is  $2n = 16$ ,  $K = 2L + 2M_1 + 2M_2 + 2M_3 + 2M_4 + 2S_1 + 2S_2 + 2S_3^+$ , the II-type is  $2n = 15$ ,  $K = 2L + 2M_1 + 2M_2 + 2M_3 + M_4 + M_4^+ + 2S_1 + 2S_2 + S_3^+$  and the III-type is  $2n = 14$ ,  $K = 2L + 2M_1 + 2M_2 + M_3 + \widehat{M_3S_3} + M_4 + M_4^+ + 2S_1 + 2S_2$ . And then the basikaryotypes are presumed to be as follows,  $B_1 = 8 = LM_1M_2M_3M_4S_1S_2S_3^+$ ,  $B_2 = 7 = LM_1M_2M_3M_4^+S_1S_2$  and  $B_3 = 7 = LM_1M_2\widehat{M_3S_3}M_4S_1S_2$ . Consequently the three karyotypes may be shown as combinations of the three basikaryotypes as follows: the I-type  $B_1B_1$ , the II-type  $B_1B_2$  and the III-type  $B_2B_3$ . In these cases, the  $M_4^+$  is a  $M_4$ -chromosome with a trabant which has possibly been translocated from  $S_3^+$ . The  $\widehat{M_3S_3}$  is also a SAT-chromosome with a secondary constriction which is derived from the translocation or fusion of the  $M_3$  and  $S_3^+$  chromosomes.

In *Scirpus lacustris* ( $2n = 38, 40, 42$ ) (cf. Tanaka 1938) three different karyotypes with or without compound chromosomes (C) are observed, that is, *S. lacustris* var. *typicus* ( $2n = 38$ ),  $K = 2C + 36S$ , *S. lacustris* var. *typicus* f. *pictus* ( $2n = 40$ ),  $K = C + 39S$  and *S. lacustris* var. *tabernaemontani* f. *zebrinus* ( $2n = 42$ ),  $K = 42S$ .

1) K=karyotype, B=basikaryotype, L=large chromosome, M=medium chromosome, S=small chromosome, t=trabant.

Consequently two basikaryotypes may be detected, namely,  $B_1 = 19 = C18S$ ,  $B_2 = 21 = 21S$ . Then the three forms *typicus*, *pictus* and *zebrinus* are represented as  $B_1B_1$ ,  $B_1B_2$  and  $B_2B_2$  respectively. In these cases one compound chromosome (C) seems to be homologous with three small chromosomes (S).

In *Scilla japonica* ( $2n = 16, 18, 26, 34, 35, 43$ ) (cf. Satô 1940), various karyotypes were already found, but these polyploid series can be explained by the combinations of the two basikaryotypes,  $B_1$  with 8 chromosomes and  $B_2$  with 9 chromosomes, namely,  $K = 16 = B_1B_1$ ,  $K = 18 = B_2B_2$ ,  $K = 26 = B_1B_2B_2^{1)}$ ,  $K = 34 = B_1B_1B_2B_2^{1)}$ ,  $L = 35 = B_1B_2B_2B_2^{1)}$  and  $K = 43 = B_1B_1B_2B_2B_2^{1)}$ . In these cases, V-shaped chromosomes belonging to the  $B_1$  give the suggestion that they have been formed by translocation or fusion of the two chromosomes belonging to the  $B_2$ .

As mentioned above various basikaryotypes are found in the same species or genus, and in order to know what genetical connection exists between these basikaryotypes, the necessity is here insisted upon of observing the chromosome behavior in meiosis of the plants and their hybrids having these basikaryotypes, namely of carrying out the basikaryotype analysis.

### Basikaryotype analysis in Aloinae

In Aloinae there are three genera, namely, *Aloe*, *Gasteria* and *Haworthia* and the analysis of their karyotypes suggests the similarity or phylogenetic relationship of their basikaryotypes. In general most species are diploids ( $2n = 14$ ) although tetra-, penta- and hexaploids are also found on rare occasions. The karyotype is shown in the diploid as follows,  $K = 8L + 6S$  including generally four SAT-chromosomes. The position of the trabants is at the distal ends of the two pairs of large chromosomes in *Gasteria* and *Haworthia*, while in many *Aloe* plants it is at the distal ends of one pair of large chromosomes and at the proximal ends of one pair of small chromosomes. Four pairs of large chromosomes are distinguished according to the lengths of their short arms, namely,  $L_1$ ,  $L_2$ ,  $L_3$  and  $L_4$ . The SAT-chromosomes in *Gasteria* and *Haworthia* are  $2L_1^+ + 2L_4^+$  and those in *Aloe* have various combinations such as  $2L_1^+ + 2S_1^+$ , etc. *Aloe variegata* has a similar karyotype, including  $2L_1^+ + 2L_4^+$  SAT-chromosomes, with that of *Gasteria*.

Considering the basikaryotypes found these plants are not always homozygotic, but some are heterozygotes or hybrids. It is

1) These are also represented as  $B_12B_2$ ,  $2B_12B_2$ ,  $B_3B_2$  and  $2B_13B_1$  respectively



necessary, as already stated, for hybridization to occur if one is to pursue the phylogenetic relation among these basikaryotypes. In the interspecific or intergeneric hybrids the chromosome behavior in meiosis must be analyzed to see the state of conjugation of the chromosomes.

Three intergeneric hybrids between *Aloe* and *Gasteria* were obtained, that is, *Aloe variegata*  $\times$  *Gasteria verrucosa* var. *latifolia*, *Aloe variegata*  $\times$  *Gasteria gyūzetu* and *Gasteria gyūzetu*  $\times$  *Aloe variegata*, and the basikaryotype analysis has been carried on in these hybrids.

In the first hybrid (*Aloe variegata*  $2n = 14 \times$  *Gasteria verrucosa* var. *latifolia*  $2n = 14$ ) seven bivalents are formed in meiosis of more than half of the pollen mother cells (table 1), which show the homology of the two basikaryotypes, but two or four univalents of the large chromosomes are also observed and suggest that there is partial homology of the chromosomes. As a result of careful observation four kinds of chromatid bridges accompanied by fragments can be found. Such abnormal behavior of the bivalent chromosomes may be due to inversion of a chromosome segment which includes no kinetochore. The inversions at the distal arms of the large chromosomes release small fragments and those of the long arms near the kinetochore release large fragments. In the case of inversion when there

is a kinetochore the abnormal chromosome behavior shows a feature somewhat different from that of chromatid bridges with fragments. In practice such a case was detected by a characteristic separation of heteromorphic chromosomes of which one chro-

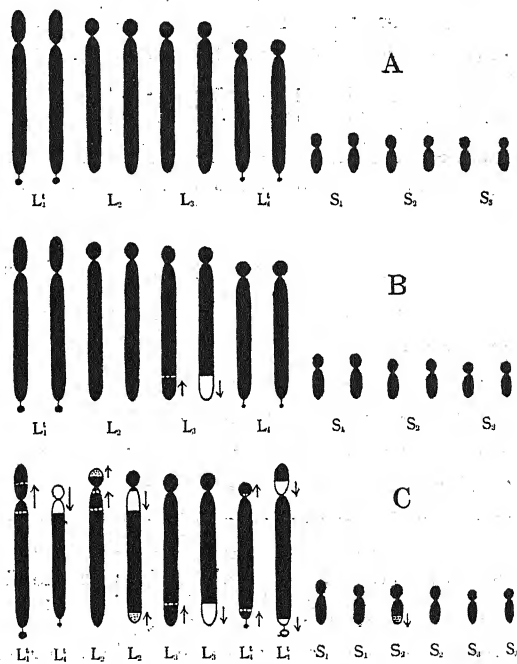


Fig. 1. Karyotypes of *Aloe variegata* ♀ (A) and *Gasteria verrucosa* var. *latifolia* ♂ (B) and their hybrid (C). Clear portions of the chromosomes represent inverted regions and dotted portions are homologous with each other. Arrows indicate direction of homologous segments.  $\times 3000$ .

matid has both the long arms of the two chromosomes. Accordingly, five kinds of inversion were analysed, these being located in four pairs of the large chromosomes. Besides these inversions one translocation was found, that is, the distal end of a large chromosome ( $L_2$ ) was homologous with the proximal end of another large  $L_2$  chromosome; moreover, this homologous region was also associated with the distal region of one of the small chromosomes. Consequently a tetrapartite consisting of the two large and one small bivalents is rarely formed. This translocation of the chromosome segments between large and small chromosomes may explain the difference in the typical *Aloe* and *Gasteria* as regards the SAT-chromosomes, that is, the

Table 1. Chromosome configurations in the first meiotic metaphases of pollen mother cells in *Aloe variegata* × *Gasteria verrucosa* var. *latifolia*,  $2n=14$ .

		I <sub>IV</sub> +5 <sub>II</sub>	7 <sub>II</sub>	6 <sub>II</sub> +2 <sub>I</sub>	5 <sub>II</sub> +4 <sub>I</sub>	Total
Preparation 1		2	125	68	4	
" 2		0	8	31	5	
" 3		1	20	7	2	
Total		3	153	106	11	273

latter in *Aloe* are  $2L_4^t+2S_1^t$  and in *Gasteria*  $2L_1^t+2L_4^t$ . It is an interesting fact that two heteromorphic large bivalents ( $L_1^tL_4^t$  and  $L_4^tL_1^t$ ) are always found in the first meiotic metaphase.

In the second hybrids (*Aloe variegata*  $2n=14$  × *Gasteria gyūzetu*  $2n=14$  and its reciprocal hybrid) partial homologous chromosomes forming a maximum of six univalents are similarly found and three inversions and one translocation are clearly observed. Besides these cases of abnormal chromosome behavior which were also found in the first hybrid, one tripartite of large chromosomes is often found. It consists of one pair of the most stable bivalents ( $L_3L_3$ ) and an  $L_1$  chromosome, judging from the chromosome configurations in meiosis (these will be reported in detail elsewhere).

Table 2. Chromosome configurations in the first meiotic metaphases of pollen mother cells in *Aloe variegata* × *Gasteria gyūzetu*,  $2n=14$ .

		7 <sub>II</sub>	1 <sub>III</sub> +5 <sub>II</sub> +1 <sub>I</sub>	6 <sub>II</sub> +2 <sub>I</sub>	1 <sub>III</sub> +4 <sub>II</sub> +3 <sub>I</sub>	5 <sub>II</sub> +4 <sub>I</sub>	1 <sub>III</sub> +3 <sub>II</sub> +5 <sub>I</sub>	4 <sub>II</sub> +6 <sub>I</sub>	Total
Preparation 1		8	2	16	6*	24	1	4	
" 2		4	1	6	0	10*	1	2	
Total		12	3	22	6	34	2	6	85

\* a univalent of a small chromosome instead of a large one was observed once in each case.

As stated above, it is postulated that by the basikaryotype analysis the occurrence of karyotype alterations such as fragmentation, fusion, inversion, translocation, deletion etc., which are presumed on the basis of the karyotype analysis to have occurred, can

be karyologically proved; moreover, it is possible to determine what regions of the individual chromosomes are homologous or non-homologous with each other, while the phylogenetic relationship of individuals or groups can to a certain extent be traced.

### Summary

The conception of the basic number of chromosomes has been established by the studies made on the chromosome numbers during the last four decades, and a basic number and morphology of the chromosomes composing it have been investigated by the method of karyotype analysis. We propose applying the term basikaryotype to such a chromosome type which is specific to individuals or groups, corresponding in number to the basic number, and viewed from the stand-point of their morphology. The chromosome behavior in meiosis has to be observed in order to pursue the investigation of the phylogenetic relation of the basikaryotypes. The criterion of the homology of chromosomes is assumed to be the pairing of the chromosomes. By this method the existence of those karyotype alterations which were either expected to have occurred or are not capable of being distinguished by the karyotype analysis may be established on karyogenetical grounds. This method is here called a basikaryotype analysis.

Comparing the basikaryotype analysis with the genome analysis, the former originated in the karyotype, while the latter started from hybridization as a premise. With the recent advances in the study of chromosome morphology the conception of the genome has come to take the karyotypes into consideration, and accordingly the new stand-point has been developed to facilitate discussion of the genetical connection of individuals or groups based upon the karyotype. The karyotype analysis is different from the genome analysis in that the former enables one to presume the relationship between the forms which can not be hybridized with each other, while it further enables determination of the basikaryotypes. The basikaryotypes may be detected in the forms in which the karyotype analysis was carried out. Some examples have been given (p. 533). The homology of the basikaryotypes may be traced by the method of basikaryotype analysis here proposed. An example of this method in the case of *Aloinae* was described (p. 534).

**Appendix:** The basikaryotype analysis can be applied in the case of forms having chromosomes of distinct morphological characteristics, while its application is difficult in the case of forms having extremely small similar chromosomes, but the conception of

partial homology of individual chromosomes which is emphasized here seems to be universally valid in the case of such small chromosomes if one adopts the conception of the secondary association developed by Darlington (1928) and his school, and other investigators (cf. Matsuura 1939).

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## Neue Beobachtungen über Protoplasmaströmung bei *Hydrilla verticillata*

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### Einleitung

Die Frage nach der Ursache der Protoplasmaströmung beschäftigt die Forscher schon seit mehr als einem halben Jahrhundert, aber da dieser Vorgang auf komplizierten inneren Vorgängen zu beruhen scheint, konnte bis jetzt selbst für Teilerscheinungen desselben keine hinreichend fundierte Erklärung gefunden werden.

Auch über den funktionären Zweck der Protoplasmaströmung liegen bis jetzt nur einige Deutungsversuche vor, die nicht durch exakte Experimente begründet sind.

Die vorliegende Arbeit vermag zwar ebenfalls keinen unmittelbaren Beitrag zur Lösung dieser Fragen zu geben, aber vielleicht kann sie zu deren weiterer Verfolgung einige Anregungen bieten, indem sie über den Einfluß einiger äußerer Einwirkungen (vor allem über die Reizwirkung von 10%iger Rohrzuckerlösung) auf die Protoplasmaströmung berichtet. Denn durch das präzise Studium der Reaktionen auf äußere Einflüsse dürfte sich am ehesten ein Weg zur Erkennung der inneren Vorgänge finden lassen.

Dieselbe Zelle reagiert auf eine und dieselbe Außenweltseinwirkung partiell verschieden. Es ist sehr schwierig, das gegenseitige Größenverhältnis des Veränderungsgrades, den die einzelnen Zellbestandteile und -funktionen Umweltreizen gegenüber annehmen, mit mathematischer Genauigkeit zu bestimmen.

So ist z.B. das Verhalten der verschiedenen Teile einer Zelle infolge der verschiedenen Permeabilität derselben gegen eine Lösung von bestimmter Konzentration verschieden, sodaß eine solche Lösung gegenüber verschiedene Zellbestandteile verschiedenen Wirkungsgrad besitzt. Bis jetzt gibt es keine Methoden, diesen spezifischen Wirkungsgrad einer Lösung von bestimmter Konzentration den verschiedenen Bestandteilen einer Zelle gegenüber zu bestimmen.

Nicht nur die verschiedenen Zellbestandteile, sondern auch der äußere und der innere Bereich des Protoplasmas zeigen verschiedene Veränderungsgrade. In den vorliegenden Untersuchungen wurde

daher die Wirkung des Reizmittels nicht als Wirkung auf das Protoplasma der Einzelzelle, sondern als Wirkung auf den Gesamtprotoplasmabestand der Versuchsobjekte studiert. Da diese Studien den Charakter einer Vorarbeit hatten, wurde auf eine mathematisch exakte Festlegung der Beobachtungen verzichtet, aber ich hoffe, daß die Ergebnisse dieser Vorarbeit von Forschern, welche den von mir beschrittenen Weg mit mathematischer Exaktheit weiterverfolgen wollen, mit Erfolg benutzt werden können.

### Beobachtungen

Zunächst wurde frisches *Hydrilla*-Material verwendet, welches mit der Hand von der anhaftenden Erde befreit und dann sorgfältig gewaschen wurde. Da dasselbe Material jeweils etwa einen Monat zur Untersuchung benutzt wurde, war diese Vorbehandlung, die das Material gegen rasche Fäulnis widerstandsfähig machte, notwendig.

Die Pflanzen wurden dann samt den Wurzeln in bedeckten Petrischalen in 10%iger Rohrzuckerlösung aufbewahrt und diese nur zur Materialentnahme jeweils kurz geöffnet. Es wurde dann immer 1 Blättchen entnommen und als Lebendpräparat in 10%iger Rohrzuckerlösung mikroskopisch untersucht.

Die Beobachtungen fingen am 10. Juni 1938 an. Im Laufe einiger Tage zeigten nur jeweils einige Zellen der immer wieder frisch entnommenen Blättchen allmähliche Veränderung. Kern und Zytoplasma bräunten sich, die Kernmembran zeigte sich partiell aufgelöst, der Kerninhalt entleert und die Protoplasmaströmung zum Stillstand gekommen. Interessant ist auch die Tatsache, daß in einigen Fällen die Protoplasmaströmung noch fort dauerte, als der Kern bereits funktionlos geworden zu sein schien.

Vom vierten Tage an jedoch konnte ich die interessante Erscheinung beobachten, daß in vielen Zellen infolge der Plasmaströmung die Kerne, wie es sonst bei den Chloroplasten und Mikrosomen der Fall ist, in passive Rotation geraten waren. Während sich der Kern entlang den Zellseiten mit ungefähr gleicher Geschwindigkeit wie die Chloroplasten fortbewegte, blieb er in den Zellecken etwas hinter diesen zurück. Dabei erlitt er fortlaufend Gestaltveränderungen, indem er sich abwechselnd verdickte und streckte. Die Rotation dauerte jeweils ungefähr 8 Stunden oder etwas länger. Bis jetzt ist über solche Erscheinungen bei *Hydrilla* nicht berichtet worden.

Die Kernrotation konnte bei *Hydrilla* auch gelegentlich an frischem Material, wenn auch nur sehr selten beobachtet werden. Aber

in diesem letzteren Falle bewegt sich der Kern viel langsamer als die Chloroplasten und die Bewegung dauerte nur sehr kurze Zeit an.

Ganz selten konnte ich auch bei frischem Material sehr rasche Kernbewegungen beobachten, aber der Kern rotierte dann nicht, sondern bewegte sich nur plötzlich sehr schnell eine Strecke weit geradeaus, um dann wieder stillzustehen.

Folgende Tabelle gibt einen Vergleich der Rotationsgeschwindigkeiten eines Kernes in einem Präparate von einem einer frischen Pflanze entnommenen Blättchen und eines Kernes in einem Rohrzuckerpräparat.

Frischpräparat	0.00057 mm/sec
Rohrzuckerpräparat	0.00820 mm/sec

Beide Messungen wurden am 17. Juni 1938, 3 Uhr nachmittags bei 24°C Zimmertemperatur und bewölktem Wetter vorgenommen. Da, wie gesagt, die Geschwindigkeit nicht konstant ist, wurde der Mittelwert der Geschwindigkeit eines vollen Umlaufes errechnet. Die Beobachtung an dem Rohrzuckerpräparat wurde 5 Stunden nach der Herstellung desselben vorgenommen. Zu der Zeit, da die Kerne diese bemerkenswerte Bewegung ausführten, erschienen die betreffenden Zellen besonders klar und hell. Nicht nur örtlich, sondern auch zeitlich war die Kerngeschwindigkeit inkonstant. Der Durchschnittswert steigerte sich allmählich und kontinuierlich mit der Zeit, erreichte schließlich ein Maximum und sank dann ebenso kontinuierlich allmählich wieder auf Null ab.

Auch Umwelteinflüsse wie Licht, Temperatur, Sauerstoffgehalt hatten bedeutenden Einfluß auf Beginn und Geschwindigkeit der Kernbewegung.

Die Zahl der obenbeschriebenen abgestorbenen Zellen nahm zwar täglich zu, aber es konnten doch bis zum 24. Juni, dem 10. Tage nach Beginn der Kernrotation rotierende Kerne beobachtet werden. Am 28. Juni zeigten nur noch die Ränder der Zellen der Blätter von den äußersten Zweigen der Pflanze lebendes Plasma,<sup>1)</sup> von dessen Chloroplasten sich nur mehr ein Teil und dieser sehr langsam bewegte. Da ich mit einem ziemlich raschen Absterben der ganzen Pflanze in der starken Rohrzuckerlösung gerechnet hatte, war ich über diese lange Lebensfähigkeit sehr erstaunt. Darum stellte ich zum Vergleich Beobachtungen über die Lebensdauer in reinem Wasser an und fand, daß ein abgetrenntes Blatt darin einen ganzen Monat am Leben blieb. Früher habe ich in unserem

1) Was darauf schließen läßt, daß die Zuckerlösung nicht durch die Epidermis sondern nur auf dem Wege durch die Leitungsgegewe eindringt.

Institut ähnliche Versuche mit *Nitella* gemacht und bis zum 43. Tage Protoplasmaströmung beobachten können. In diesem Falle handelte es sich allerdings nicht um ein einzelnes Blatt, sondern um ein Zweigstück mit mehreren Blättern.

Es sei noch festgestellt, daß bei *Hydrilla* 10% die Grenzkonzentration für die Plasmolyse durch Rohrzucker darstellt.

Bei *Vallisneria* hat FITTING einmal die interessante Beobachtung gemacht, daß bei Chemodinese Kern und Mikrosomen Rotation ausführen, während die Chloroplasten stillstehen—eine Erscheinung, für die bis heute keine Erklärung gefunden werden konnte.

Beachtenswert ist in diesem Zusammenhang noch, daß bei frischem Material die Kernbewegung durchaus nicht immer der Bewegung des Plasmas folgt, sondern daß der Kern sich oft in anderer, ja sogar entgegengesetzter Richtung bewegt.

Wassermangel kann nach unseren Versuchen nicht als Ursache der Kernrotation angesehen werden. Deshalb ist für die Erklärung von meinen und FITTINGS Beobachtungen wohl Reizwirkung als Ursache anzunehmen. (Während dieser Beobachtungen war eben das Wetter fast immer bewölkt.)

Außerdem konnte ich im Sommer 1939 an *Hydrilla* noch andere interessante Beobachtungen über die Protoplasmaströmung anstellen.

Bisher galt die Richtung der Protoplasmaströmung in ein- und derselben Zelle für unveränderlich. Nach meinen Beobachtungen ist es, wenn auch selten, doch mit Sicherheit möglich, die Richtung der Protoplasmaströmung umzukehren.

Ich ließ frische *Hydrilla* mehrere Stunden auf dem Tisch liegen und brachte sie dann wieder ins Wasser. Fast alle Teile der Pflanze erwiesen sich dann als abgestorben, nur die Blätter der Spitzenteile waren (ähnlich wie in dem früher beschriebenen Falle) noch lebendig. Wurden von diesen Spitzenblättern Rohrzuckerpräparate hergestellt, so begann sich in einigen Zellen derselben, selten bereits nach 1 Stunde, meistens erst nach 4 Stunden, eine der bisherigen entgegengesetzte Plasmaströmung zu entwickeln, die allmählich, während die ursprüngliche immer mehr zurückging, vorherrschend wurde und schließlich das ganze Zellplasma beherrschte. Wenn diese Strömung in der entgegengesetzten Richtung zum Stillstand kam, konnte man sie wieder in Gang bringen, indem man dem Präparat einige Tropfen Wasser zufügte. Ganz selten ist auch in frischem Material diese Umkehrung der Strömungsrichtung zu beobachten.

Oft zeigten auch unter gleichen Bedingungen Zellen anfänglich Zirkulation, welche dann unter sehr deutlicher Übergangserscheinung



sich allmählich in Rotation verwandelte. Bewahrte man ein Wasserpräparat eines frischen Blattes von *Hydrilla* mehrere Tage in der Feuchtkammer oder eine ganze *Hydrilla*-pflanze unter Wasser in einer Präparatenflasche auf, so zeigte sich bei der mikroskopischen Untersuchung, daß sich die Rotationsbahn verengert hatte, zum Teil so weit, daß die rotierende Bewegung in eine penduläre übergegangen war.

Manchmal hatte sich außer der Hauptrotationsbahn noch eine besondere, kleine, kreisförmige gebildet, welche, langsam die Zelle durchwandernd, ihren Ort allmählich veränderte. Außerdem waren in dem veränderten Umweltbedingungen (Rohrzuckerlösung, Austrocknung-) unterworfenen Material noch verschiedene andere, wenn auch nicht so ausgeprägte Abweichungen von der normalen Strömung zu beobachten.

### Zusammenfassung

1. Unter der Reizwirkung von 10%iger Rohrzuckerlösung wird bei einzelnen Zellen auch der Zellkern unter Gestaltveränderungen mit wechselnder Geschwindigkeit durch die Protoplasmaströmung mitbewegt.

2. Diese Kernrotation beginnt je nach den Umweltbedingungen (Licht, Temperatur, Sauerstoffgehalt etc.) nach verschieden langer Zeit, aber in diesem Falle am 4. Tage nach Beginn der Einwirkung der Zuckerlösung, und dauert ca. 10 Tage an.

3. In einigen Fällen wurde beobachtet, daß die Protoplasmaströmung noch fort dauerte, als der Kern bereits abgestorben zu sein schien.

4. Bei Wiederbenässung von ausgetrocknetem *Hydrilla*-material tritt in einem Teil der noch lebenden Zellen Umkehrung der Rotationsrichtung ein.

5. In Wasserpräparaten von *Hydrilla*-blättern, welche mehrere Tage in der Feuchtkammer aufbewahrt worden waren, ging die rotierende Bewegung zeitweise in eine penduläre über. Auch andere Abweichungen von der Normalströmung waren zu beobachten.

Zum Schlusse möchte ich noch meinen verehrten Lehrern, den Herren Ehrenprofessor FUJII und Prof. NAKANO der Kaiserlichen Universität Tokyo für ihre gütige Anregung bei dieser Arbeit meinen verbindlichsten Dank aussprechen. Ebenso möchte ich der Japanischen Gesellschaft zur Förderung der Zytologie für ihre finanzielle Unterstützung meinen besten Dank aussprechen.

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## Extrusion of a Nucleolus *in toto*, Found in the Ovarian Oocytes of *Holothuria monacaria*

By

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Received March 13, 1940

*Holothuria monacaria* were collected at the Misaki Marine Biological Station in the summer of 1929, and their gonads were fixed in Bouin's solution. Upon examination of the preparations made from ovarian tubules, not infrequent cases were found of a large nucleolus being extruded as a whole from the nucleus into the cytosome of growing oocytes (Figs. 6-9).

Many observers have hitherto held the view that the discharge of nucleolar fragments into the cytosome of young oocytes and of somatic cells surely occurs in various species and the yolk-substance, fat, mucin, pigment, and zymogen-granules may probably be derived from these discharged fragments of nucleoli.<sup>1)</sup> Nevertheless, it was found that none of the authors has ever caught any case of nucleoli which are in the process of being extruded from the nucleus into the cytosome.

The present report deals mainly with the successive processes of this phenomenon as found in the ovarian oocytes of *Holothuria monacaria*. The fate of the extruded nucleolus in the cytosome is not noted here.

### Ovarian Oocytes

The ovarian oocytes, at every stages of development, are loosely enclosed in an ovarian tubule, and each oocyte is enveloped in a follicle membrane to which it is attached by a stalk, the canal of the so-called micropyle of the oocytes (Fig. 6). In the canal of this micropyle was found a curious chromatoid fibrillar structure as is shown in figure 6.

1) See, Wilson's Cell, 1925: 345-346, 353-355; Berg, W. 1932 Über den Übertritt von Kernstoffen in das Cytoplasma. Zeit. f. mik. A. Forsch. 28: 565; Ito, Toshio 1938, Über die Formveränderung der Randnukleolen der wachsenden Oozyten bei einem Knochenfisch mit besonderer Berücksichtigung auf die Frage über den Austritt der Nukleolarsubstanz ins Zytoplasma. Cytologia, 6: 283. Oka, T. 1931. On the accidental hermaphroditism in *Oryzias latipes*. Journ. Fac. Sci. Imp. Univ. Tokyo, Sect. IV, 2-3: 219-224.

Growing oocytes have an expanded germinal vesicle in which generally only one large, round chromatin nucleolus is found. In some oocytes, however, there are found two nucleoli, one of which is larger than the other, in the same germinal vesicle (Fig. 5). The chromatin nucleolus stains heavily with Delafield's and Heidenhain's hematoxylin, but sometimes it stains faintly with eosin and other acidic dyes.

A small nucleolus is round in shape, containing scarcely any vacuole and stains uniformly. A larger nucleolus contains several vacuoles, and is irregular in contour, because of peripheral vacuoles (Figs. 1 and 5). It should be noted that in the cytosome of oocytes, there are found one or two large oval discharged nucleoli, containing so many vacuoles that their contour is irregular. These extruded ones stain faintly both with basic and acidic dyes. In addition to the extruded nucleolus, there are found in the cytosome several minute granules which stain with basic dyes.

### Extrusion of Nucleolus into the Cytosome

As a rule, the germinal vesicle of this species contains one small nucleolus in the early stages of primary oocytes. As the germinal vesicle grows larger, a nucleolus is extruded into the cytosome, then a young one appears in the germinal vesicle in place of the discharged one (Fig. 9). In not rare cases, there are found two nucleoli, a larger and a smaller one (Fig. 5).

Successive stages of extrusion of a nucleolus *in toto* from the germinal vesicle into the cytosome can easily be traced, especially in such young oocytes as those in the ovarian tubules fixed in late summer. The germinal vesicle of these oocytes measured about 35.5 microns in diameter (Figs. 1-9).

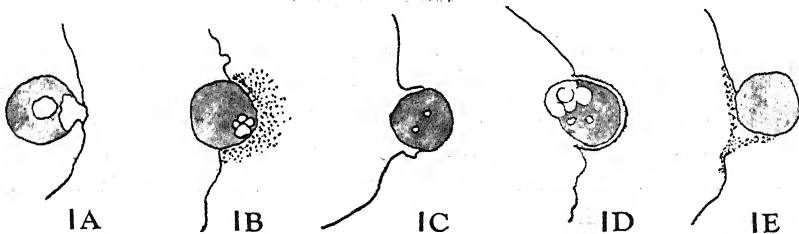


Fig. 1, A-E. Successive stages of the extrusion of a nucleolus *in toto* from the germinal vesicle into the cytosome of Holothurian oocytes. The nucleolus is about 7.5 microns in diameter, while the growing germinal vesicle at this time is about 35.5 microns.  $\times 900$ .

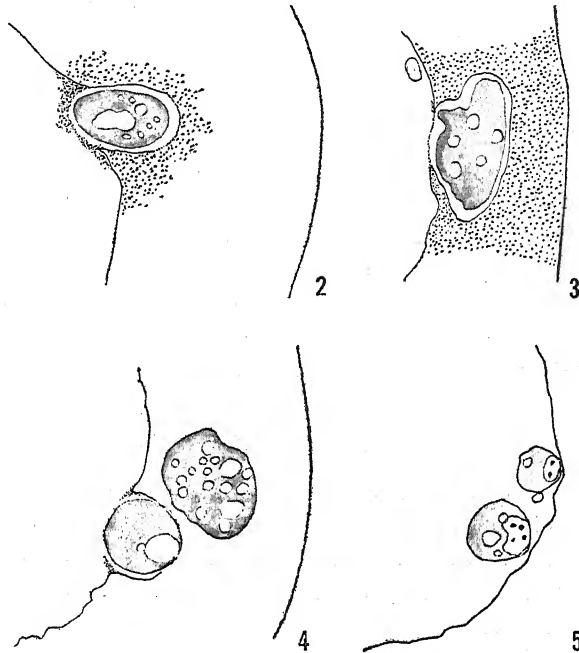
A large nucleolus that is half or one-third at the way through the membrane of the germinal vesicle was often found (Figs. 7-9).

Figures 1E and 3 show a nucleolus which has been extruded and is situated just outside the germinal vesicle.

So far as I know no other cases of the extrusion of a nucleolus *in toto* has ever been observed or described before.

In general, the extrusion of a nucleolus commences when it has grown of about 7.5 microns in diameter, (the germinal vesicle of the oocytes at this stage is about 35.5 microns in diameter). It should be remembered that the germinal vesicle grows continuously larger as cytoplasmic maturity is attained.

The nucleolus which is about to be extruded from the germinal vesicle contains so many enlarged vacuoles that it looks somewhat like a morula. Such a nucleolus comes to lie nearly in contact with the membrane of the germinal vesicle. It should be noted here

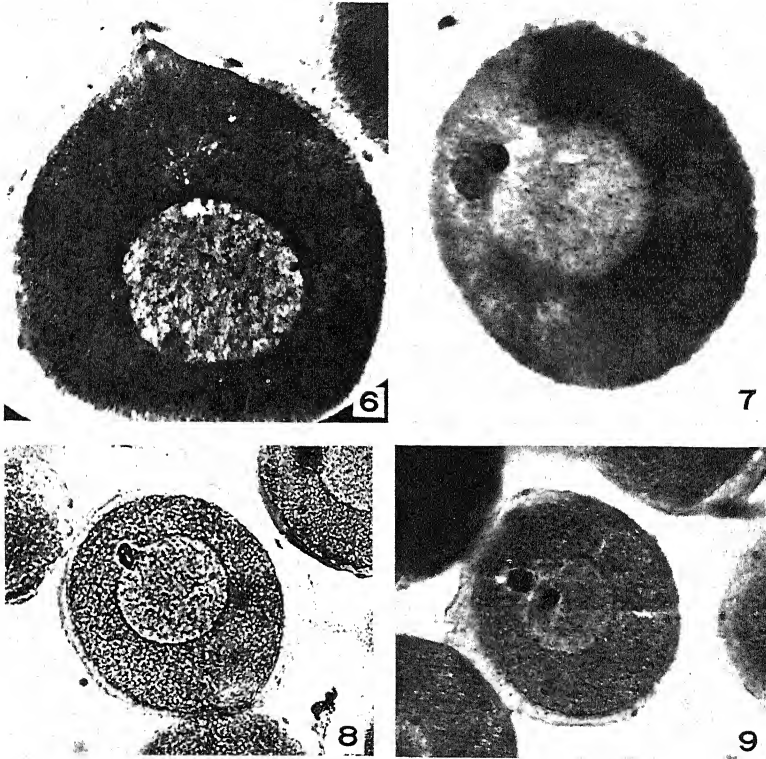


Figs. 2-5.  $\times 900$ . A nucleolus just extruded from the germinal vesicle, and over the mouth of the membrane pocket dented deep into the cytosome there has been established a new covering membrane of the germinal vesicle. {3. A nucleolus just extruded into the cytosome, being configured to amorphous in shape. 4. {A round nucleolus that is about to be extruded from the germinal vesicles, and an amorphous nucleolus that has been extruded into the cytosome, the two being found in the same oocyte. 5. A part of the germinal vesicle containing an old and a young nucleolus.

that the germinal vesicle at this stage is not in a completely expanded state, but has shriveled up a little as is shown in figures 1-5. That part of the membrane of the germinal vesicle to which a nucleolus has approached is gradually dented outward, and the part becomes a pocket in which a morula-like nucleolus is enclosed (Figs. 1, 8, and 9). The nucleolus at this stage is not necessarily oval in shape (Figs. 1D and 2), but is round in some cases (Figs. 1B and 4). The vacuoles contained in such a nucleolus are found sometimes

directed to the membrane (Figs. 1A and 4) and in other cases they are situated at the opposite side from the membrane (Fig. 1D).

There is hardly any space between the anterior hemisphere of the enclosed nucleolus and the membrane (Figs. 1 and 4), but space remains between the posterior hemisphere of the nucleolus and the nuclear membrane (Figs. 1 and 4).



Figs. 6-9. Photomicrographs of growing oocytes of *Holothuria monacaria*. 6. A growing oocyte, in which the stalk of so-called mikropyle (mp) and the chromatoid fibrillar-structure is found in the canal of mikropyle. An extruded nucleolus and a young one in the germinal vesicle are seen.  $\times 670$ . 7. In a growing oocyte a round nucleolus heavily stained is found extruding two thirds way from the germinal vesicle, and an oval nucleolus extruded is in the cytosome.  $\times 670$ . 8, 9. Growing oocytes, in which a nucleolus is extruding from the germinal vesicle.  $\times 420$ .

As the process of protrusion of the pocket containing the nucleolus goes on, the mouth of the pocket becomes gradually narrower and narrower, and finally the opening is closed. This closure is brought about by an accumulation of minute particles (Figs. 1E-4). Now the nucleolus enclosed in the pocket has been excluded completely from the germinal vesicle (Figs. 1E and 3).

Soon after the extrusion is completed, the membrane of the pocket that enveloped it is dissolved, and there appears a clear zone between the extruded nucleolus and the cytoplasm in which it is imbedded (Fig. 3). The space is eventually filled with cytoplasm (Fig. 4).

It is worthy of notice that the extrusion of nucleoli occurs successively several times, and a young nucleolus is formed anew in the germinal vesicle when a fully grown nucleolus is discharged into the cytosome, and so in not rare cases, two nucleoli, an old and a new one, are found in one germinal vesicle (Fig. 5).

### Extruded Nucleolus

Vacuolization becomes more active in the nucleolus extruded into the cytosome, and its outline becomes irregular in shape because of its peripheral vacuoles (Figs. 3 and 4). Such an extruded nucleolus stains with eosin in ordinary cases.

Beside these, there are found several minute chromatoid bodies which stain with Delafield's haematoxylin can be seen in oocyte cytosomes.

Drs. K. and J. C. Dan, of the Misaki Marine Biological Station, were kind enough to read through the manuscript.

### Summary

1. Remarkable cases of the extrusion of a nucleolus *in toto* from the germinal vesicle into the cytosome of ovarian primary oocytes of *Holothuria monacaria* were studied cytologically, and all stages of the process were traced.

2. Primary oocyte of this species has one chromatin nucleolus, which grows as large as about 7.5 microns in diameter, becomes vacuolized and finally is extruded from the germinal vesicle when it has attained a size of about 35.5 microns in diameter. A newly formed young nucleolus is found growing in the germinal vesicle when an old one is extruded.

3. The extrusion of a nucleolus takes place successively several times in the growing oocytes.

4. The nucleolus that is about to be discharged contains several vacuoles, and comes to lie close to the membrane of the germinal vesicle which now becomes slightly shriveled.

5. That part of the membrane where an extruding nucleolus attaches comes to be dented outward, and then becomes a pocket containing the nucleolus, protruding into the cytosome.

6. After the pocket containing the nucleolus has been protruded enough into the cytosome, a new membrane is formed covering the mouth of the pocket.

7. Vacuolization of the nucleolus becomes more pronounced after it has been discharged into the cytosome, and then it becomes a large, amorphous mass which stains with acidic dyes.

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**Cytogenetic Studies in Artificially Raised Interspecific Hybrids  
of *Papaver*. IX. On the bivalents-association in the  
meiosis of the PMC of *Papaver somniferum*<sup>1)</sup>**

By

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(With 6 figures)

*Received April 1, 1940*

**Introduction**

In previous papers (YASUI 1936, 1937a, 1937b) I have presented the view that the chromosome constitution of *P. somniferum* is  $2(4_{II}+3_I)$ , and that of *P. orientale* is  $2(7_{II}+7_I)=2(O_1O_2+O_3)$ , and also that there may be homologous chromosomes among those of  $O_1$  and  $O_3$  and of  $O_2$  and  $O_3$  in *P. orientale*.<sup>2)</sup> Further consideration on these points led me to the reexamination of the chromosome behaviour in these parent plants, and I have found some evidence in the chromosome associations to justify the chromosome constitution mentioned above. In this paper I should like to limit myself to the description of *P. somniferum*.

**Material and Method**

Meiotic chromosomes in the PMC were investigated in aceto-carmin smear materials and also the paraffin sections. The latter were fixed with NAVASHIN's fluid and BENDA's modification of FLEMMING's fluid. HEIDENHAIN's iron-alum haematoxylin was mostly used for the staining of the paraffin sections. Photos were taken with the aid of ZEISS H. I.  $\times 100$  and LEITZ periplane ocular  $\times 10$ . Drawings were made with a camera lucida.

**Investigation**

1. **The PMC.** *Papaver somniferum* has 22 somatic chromosomes. In one race of this plant, there appeared in the diaphase of the 1st meiotic division of the PMC 11 bivalents. Of the latter, 6 formed rings, 2 made side by side pairings or open rings, one showed end to end union, one showed a small open ring and the remaining,

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 252.

2) Cf. YASUI 1936, p. 540.

largest one showed side by side or open ring configuration, while in an other race almost all the bivalents showed closed or open rings. The component chromosomes of the largest pair (I) associate at one end with each other and attach with other ends having satellites to the nucleolus (Fig. 3, *a*). Two large ring bivalents (II and III) attach themselves to the same nucleolus to which the bivalent I attaches (Figs. 1c, *b*, *c*, and 5c, *b* and *c*). Other 2 ring bivalents (VII and VIII), which are smaller than the bivalents II and III, always associate with each other in the diaphase (Figs. 1c, 2, 3, 5c and 5d, *f* and *g*). These two pairs of the bivalents are often interlocked with each other though they do not show any chiasma formation between the arms of the companion sets (Figs. 2, *c*, *b*; 3, *f* and *g*; 5c, *b* and *c*; 5d, 5e, *f* and *g*). Another pair of bivalents (IV and V), which show generally the side by side association but sometimes open rings, also associate (Figs. 1c, 2, *d* and *e*). In some cases the latter join at the closed ends of the bivalents (Fig. 4, *d* and *e*). Such may be the result of interlocking. One bivalent (XI) (Fig. 1c, *i*) showing the end to end association almost always appeared very near to a ring bivalent (IX) (Fig. 1c, *h*).

In another race there is no bivalent which associates end to end, but instead an open ring which associates with another open ring bivalent, so that in this race there are 4 pairs of ring bivalents. The remaining 2 bivalents (VI and X) (Figs. 1c, 2, 5d, 5e, *j*, *k*) always behave as solitaires showing no sign of association, not only between themselves but also with other bivalents. Thus we can recognize 3 sets of chromosomes in the haploid complement of *P. somniferum*. Two of them ( $S_1$  and  $S_2$ ) resemble each other while the third ( $S_3$ ) has no chromosomes homologous to those in the other two sets.\*

In the metaphase of the 1st meiotic division those associated bivalents separated generally from each other, though in some rare cases they came together at the equator showing the pairings. Here I wish to propose the term "bivalents-association" for such union of bivalents without chiasma formation, and it is an association of two bivalents as a whole in the later prophase of the meiotic division.

**2. Root tip cell.** The karyotype of *P. somniferum* seems not to be simple because *P. somniferum* consisting of a number of races, seems to have several different karyotypes among them. But as we see in the figure 6, it has some sets of similar chromosomes, though they do not show a perfect resemblance. An example of the chromosome diagnosis is shown in Table 1.

\* Hereafter we wish to use  $S_1$ ,  $S_2$  and  $S_3$  to denote chromosome sets of 4, 4, and 3 in *P. somniferum*.

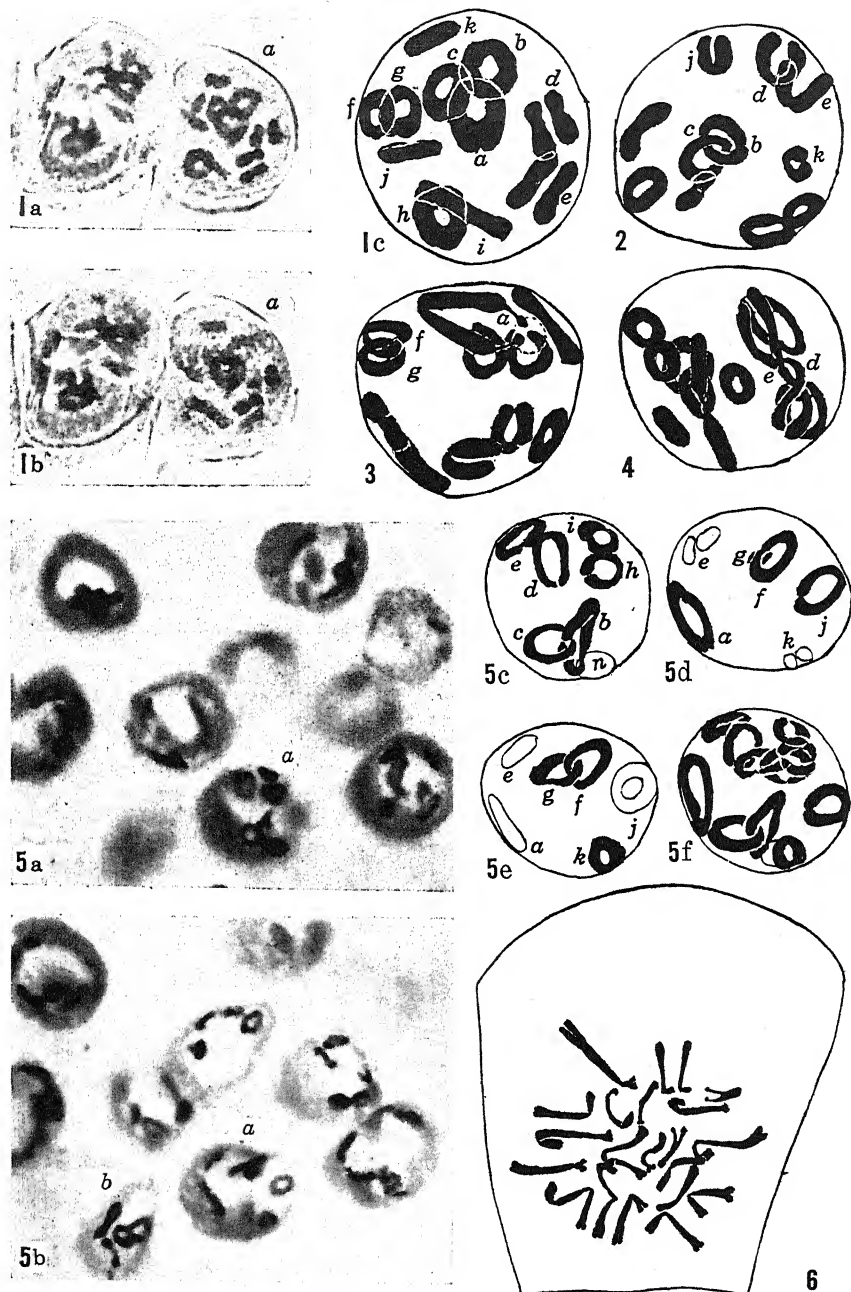
Chromosomes I-X have their homologous partner in the somatic cell, but  $XI_1$  and  $XI_2$  have no exactly homologous partner, while they are like each other except that  $XI_1$  has 2nd constriction in one arm, while  $XI_2$  has none. These two may associate in the meiosis to form a geminus.

Table 1. Chromosome constitution in a race of *P. somniferum*

Kinds of chromosomes	Length	Position of the 1st constriction	Position of satellite and the 2nd constriction
I	Long	Submedian	A satellite at the distal end of the short arm, 2nd constriction in the distal part of the long arm
II	Long	Subterminal	A satellite at the distal end of the short arm, 2nd constriction at the middle part of the long arm
III	"	"	"
IV	Long	Subterminal	
V	"	"	
VI	Median	Subterminal	A satellite at the distal end of the short arm
VII	Median	Submedian	A satellite at the distal end of the short arm
VIII	"	"	"
IX	Median	Subterminal	A satellite at the distal end of the short arm, 2nd constriction at the distal part of the long arm
X	Median	Subterminal	
$XI_1$	Median	Subterminal	A satellite at the distal end of the long slender arm, 2nd constriction at distal part of the other arm forming a round knob
$XI_2$	Short	"	A satellite at the distal end of the long slender arm, no 2nd constriction as in the $XI_1$

We can see in Table 1, that in 3 pairs of chromosomes, i.e. II & III, IV & V, and VII & VIII, the corresponding partners very much resembled each other. The resemblances in their shape and size led me to the idea that these pairs may correspond to those which form bivalents-associations II & III, IV & V, and VII & VIII respectively. The bivalent consisting of  $XI_1$  &  $XI_2$  (XI) may associate with the bivalent IX to form bivalents-association IX & XI.

In the center of the nucleus in Fig. 6 we see 3 chromosomes which associate intimately. They are from left to right  $IX_1$  and  $IX_2$ , and  $XI_1$ . They always appear very near to each other, while  $XI_2$  does not come together with  $XI_1$  in the somatic division. In this somatic association the nucleolus plays a certain role, but in some other somatic associations of the resembling chromosomes which generally occur in this species no clear relation with the nucleolus was found.



Figs. 1-5. PMC in the diaphase of *Papaver somniferum*. 1-4 from aceto-carmine smear, 5 from a paraffin section, 1a and 1b, one and the same material photographed in different levels.  $\times 1000$ . 1c-4, camera lucida drawing. 1c, one and the same PMC with 1a, 1b, a; a, bivalent I; b and c, ring bivalents II and III; d and e, bivalent IV and V; showing side by side association; f and g, ring bivalents VII and VIII; h, ring bivalent IX; i, bivalent XI, showing end to end association;

## Discussion

1. **Bivalents-association.** There are cases of the chromosome association in the meiotic division other than the normal pairing of homologous chromosomes to form gemini. KUWADA (1910) in *Oryza*, TAHARA (1910) in *Morus* and ISHIKAWA (1911) in *Dahlia* have found the association of metaphase chromosomes in the II meiotic division. DARLINGTON (1928) described a similar association in the 1st meiotic metaphase of *Pyrus* and proposed the name "secondary pairing." He has considered it as the pairing of small homologous chromosomes due to some effect of fixation. After that, LAWRENCE (1931) discussed the secondary association thoroughly, and used the latter as the criterion of the homology of the chromosomes. According to DARLINGTON and LAWRENCE the secondary association is the union of the homologous chromosomes of small size in metaphase which failed to form multivalents in the meiotic prophase. The chromosomes in the secondary association do not show any differences either in their size or shape, so it is different from the writer's bivalents-association, which appear in the later prophase of the 1st meiotic division, but does not appear in the metaphase or later. Chromosomes in bivalents-association show clear differences among themselves not only in their size but also in shape. They may be considered as very loosely associated quadrivalents. For the formation of the bivalents-association, on one hand the so-called matrix (hyalonema of FUJII) of chromosome may have a certain role, while on the other hand we have to consider the influence of the nucleolus. In our investigation the bivalents I-III attach to one and the same nucleolus, where they may have not only the opportunity of association but also may absorb the same material from the nucleolus which have some part in the development of the chromosomes. But in this case the bivalents-association occurs not only in the SAT-chromosomes but also between the bivalents which do not show any sign of having the satellites or second constrictions. Consequently the nucleolus may not be the main cause of bivalents-association.

Here I wish to mention that bivalent I in the chromosome set

*j*, open ring bivalent VI; *k*, smallest open ring bivalent X; 2, a PMC in which *c* and *b* show the interlocking of bivalents II and III; *d* and *e*, open ring IV and V; 3, a PMC. *a*, bivalent I showing satellites very clearly; *f* and *g*, bivalents VII and VIII showing the interlocking; 4, a PMC. *d* and *e*, interlocked bivalents IV and V at the distal ends. ca.  $\times 2000$ . 5a and 5b, one and the same material, photographed in different level.  $\times 1000$ . 5c-5e, camera drawings in different levels of the nucleus in one and the same cell (*a*) as 5a and 5b; 5c, shows 3 bivalents associations of ring bivalents; 5d and 5e show interlocked bivalents associations of ring bivalents (*f*, *g*); 5f, a nucleus reconstructed from 5c, 5d and 5e; ca.  $\times 1750$ ;

Fig. 6, a root tip cell showing a polar view of an equatorial plate. ca.  $\times 1750$ .

$S_3$  may bear some resemblance to bivalents II and III in other 2 sets,  $S_1$  and  $S_2$ .

2. **Derivation of the chromosome complement of *P. somniferum*.** In previous papers (YASUI 1936, 1937a, and 1937b) I have stated that *P. somniferum* has 3 sets of chromosomes in its haploid complement. One of them consists of 3 chromosomes, and the two of similar sets of 4 chromosomes. So that the chromosomes in the latter two sets form bivalents when the haploid complement of *P. somniferum* loses its partner in the meiotic division, e.g. in the interspecific hybrids of *P. somniferum*, which was crossed with other species having no homologous chromosomes. The present investigation confirms the above consideration. *P. somniferum* has the haploid complement which consists of 3 sets of chromosomes, two of them ( $S_1$  and  $S_2$ ) greatly resemble each other and the third ( $S_3$ ) has no homologous chromosomes in the other 2 sets, except that one chromosome (I) in set  $S_3$  attaches to one and the same nucleolus on which chromosomes II and III in the  $S_1$  and  $S_2$  sets attach.

The following three considerations may be given for the derivation of such a chromosome complement. 1. a hybrid whose haploid chromosome number is 7 was raised as an amphidiploid by the intercrossing of 2 species in which one has 3 haploid chromosomes and the other 4 haploid chromosomes; then this amphidiploid was crossed with another species closely related to one parent having 4 haploid chromosomes, and again all the chromosome sets were doubled forming an amphitriploid, namely  $(3+4) \times 2 = 2(7)$ , and then  $(7+4) \times 2 = 2(4+4+3) = 2(11)$ . 2. the chromosome complement in a species having 4 chromosomes as its haploid number was doubled and then crossed with a species having 3 haploid chromosomes, and again all the sets were doubled, namely  $2(4+4) = 2(8)$ , and then  $(8+3)2 = 2(11)$ . 3. in an interspecific hybrid one of whose parents has 4 haploid chromosomes and another 3 chromosomes, the sets of 4 chromosomes was doubled and again the whole sets were doubled, namely  $4+3$ , and then  $2[2(4)+3] = 2(11)$ .

As I have mentioned above, the members of the bivalents-association are not completely homologous and neither can take the other's place in the meiotic division in the parent species. Such differences in every set of 4 may be difficult to be derived by doubling of one set of 4 chromosomes, so that it looks to me that the first consideration is more possible.

### Summary

1. In a race of *Papaver somniferum* are found 11 bivalents in its diaphase of the PMC, which show the kinds of association as 6

rings, 2 side by side union or, rarely, 2 open rings, 1 end to end union, 1 small ring and 1 largest side by side union or open ring. While in another race there were found only ring or open ring configurations instead of side by side or end to end unions.

2. There were observed in the former very loose associations "bivalents-associations," between 2 pairs of ring bivalents or one pair of side by side bivalents, and also between a ring and an end to end bivalent. In the latter race 4 associations of ring bivalents were found.

3. *P. somniferum* is an amphitriploid, which has probably been derived as follows: an interspecific hybrid is raised between 2 species, one having 4 chromosomes and the other 3 chromosomes as their haploid complements, then the chromosome sets were doubled causing an amphidiploid, namely  $2(4+3)=2(7)$ . This amphidiploid is crossed with a plant which is closely related to one parent having 4 haploid chromosomes, then the whole sets are doubled again, namely  $(3+4)2=2(7)$ , and then  $2(7+4)=2(11)$ .

4. The association or pairing of the two bivalents, "the bivalents-association" was discussed.

The writer's hearty thanks are due to Prof. K. Fujii for his kind advice in the course of the investigation. The expence of carrying out the present work was partly defrayed out of a grant from the Japan Society for the Promotion of Scientific Research, to which I tender my thanks.

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## Chromosome Studies on Papaveraceae with Special Reference to the Phylogeny \*

By

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Received April 19, 1939

Although there are about 600 species of Papaveraceae, there have been chromosome studies made of comparatively few of them, in spite of the majority of them being cultivated in the gardens.

It is hoped therefore that it will be of some interest to describe some of the chromosome studies of Papaveraceae which the writer has carried on during the last few years, especially with a view to find the phylogenetic relationships between various genera of this family.

The first karyological studies of the Papaveraceae were made by Němec (1910) on *Corydalis pumila* and later by Tahara on *Papaver rhoeas*, *orientale* and *somniferum*. Afterwards Ljungdahl (1922-'24), Yasui (1921-'39) on *Papaver*, Winge (1925) on *Eschscholtzia*, Kachidze (1926) on *Platystemon* were the chief ones on karyological studies in Papaveraceae.

Here the writer wishes to express his best thanks to the directors of the botanical gardens at Berlin-Dahlem, Graz, Kaunas, Kew, Leyden, Oslo, Paris and Warsaw for the seeds that were kindly sent to him for the present study.

### Material and Method

Only young flower buds were collected for study. The Farmer's aceto-alcohol was used for fixation and Heidenhain's iron-alum haematoxylin for staining in order to be able to compare the present preparations with the former ones, upon which my previous descriptions on chromosomes of higher plants are based.

### Plants Investigated <sup>1)</sup>

#### Hypecoideae

According to Fedde (1936) there are two genera *Pteridophyllum* and *Hypecium*.

\* This work was made possible by a grant from the Japan Society for the Advancement of Cytology, to which society the writer's most appreciative thanks are due.

1) The classification in this family is based chiefly on F. Fedde's "Papaveraceae" in "Pflanzenfamilien", 2nd Ed. 1936.



*Pteridophyllum racemosum* (Fig. 1). It is only grown wild in our country.

The outer and inner morphological characters in this plant are quite different from those of the latter genus. Murbeck (1912), therefore, has established a new subfamily Pteridophylloideae, and Hayata (1930) also adopted a new family Pteridophyllaceae for the reason that they had simpler stele and different floral structure from those of other Papaveraceae. Prof. Nakai of the Tokyo Imperial University holds the same opinion.

Karyological results lead to the same conclusion. Its meiotic number 9, which is almost never found (except in *Roemeria*) in this family, must be derived from 3, the basic number of Papaveroideae (the writer's Cheridonioideae, cf. p. 574, phylogenetic scheme) and trebled. Murbeck said "*Pteridophyllum* ist auf Grund seiner isolierten Stellung unzweifelhaft ein uralter Typus, und da diese Gattung, obgleich sowohl von den Papaveroideae wie von den Hypecoideae scharf geschieden, doch eine Mittelstellung zwischen ihnen einnimmt, so könnte man sich zu der Annahme versucht fühlen, daß sich diese beiden Gruppen von dem *Pteridophyllum*-Typus abgezweigt hätten. Wahrscheinlicher ist es jedoch, daß sich die drei Unterfamilien entweder gleichzeitig aus einander sehr nahestehenden Formen entwickelt haben, oder daß die Pteridophylloideae durch ausgestorbene Gattungen einst näher mit den Papaveroideae verbunden gewesen sind und also eine frühzeitige Auszweigung von diesen darstellen".

According to Fedde's description no seeds are seen in this plant. It may be so, as polypory is observable in it.

The *Pteridophyllum*, being different from other papaveraceous plants not only in outer appearances but also as regards inner anatomical and karyological structure, as stated above, should be established as an independent family Pteridophyllaceae. It is probable that *Pteridophyllum*, having 9 meiotic chromosomes, branched out from very remote ancestors of Papaveroideae (the writer's Cheridonioideae, cf. p. 574, phylogenetic scheme) with the basic chromosome number 3, and has now grown quite stable by polyploidation.

*Hypecoum procumbens* (Fig. 2). This plant, being different from the previous one both as regards its outer and inner morphological structure, has 8 meiotic chromosomes. Smith (1934) counted, however, 12 somatic chromosomes. In spite of the size of the meiotic chromosomes being rather larger than those of *Pteridophyllum*, as stated above, the pollen mother cells are far smaller

than that, the former  $7.5\mu$  in diam. and the latter about  $14\mu$  in diam. The basic number in this genus is 4. From the basic number 4 in this genus and 3 in the above mentioned species, it is considered that they have been developed face to face in each other as shown in the phylogenic scheme (cf. p. 574).

### Papaveroideae Platystemoneae

*Platystemon californicus* (Fig. 3). There has been still no report about the chromosome numbers in this genus. Kachidze (1926) communicated to S. Nawashin that it had 6 meiotic chromosomes. The writer has counted 6 also. The pollen mother cells and meiotic chromosomes are larger than those of *Hypercium procumbens*. We agree with Dickson's opinion that the present genus is the most primitive one in the Papaveroideae (the writer's Chelidonioideae, cf. p. 574, phylogenic scheme).

*Hunnemannia fumariaefolia*. (Fig. 4) The genus *Hunnemannia* has only one species which is half xerophytic. It was originally grown wild in Mexico. The writer counted 28 meiotic chromosomes in 1931. The same number was also counted by Smith (1934).

The first metaphase chromosomes, being ellipsoidal, are rather small in comparison with those of other Papaveroideae (the writer's Chelidonioideae and Papaveroideae), while the pollen mother cells are very large, about  $25\mu$  in diam. From the results of my karyological observation in Papaveraceae it can be said that these pollen mother cells are the largest of the papaveraceous group. The basic number in this genus may be 7. Probably this plant arose as an effect of crossing between some species like *Corydalis* or *Dicentra* (Corydaloideae cf. 574 phylogenic scheme) and some *Eschscholtzia* (Chelidonioideae cf. p. 574 phylogenic scheme) in very early times.

The above suggestion seems to be substantiated because the geographical distribution of above 3 species is almost the same.

*Eschscholtzia pulchella*. (Fig. 5) Formerly Winge (1925) found the meiotic chromosome number in four different species to be 6. Morinaga and alli (1929) and Lawrence (1930) have confirmed that there were 6 meiotic chromosomes in *E. californica*.

Smith (1934) has also counted 6 meiotic chromosomes in *E. caespitosa* and *E. californica*. But only Lawrence (1930) has counted eight meiotic chromosomes in *E. mollis*. The writer has also found 6 meiotic chromosomes in *E. pulchella*. The pollen mother cells are  $9\mu$  in diam. Thus, generally the basic number of chromosomes in this genus is 3.

The chromosome numbers found up to date are as follows:

Plant names	n	Authors
<i>E. aurantiaca</i>	6	Winge (1925)
<i>E. californica</i>	6	Lawrence (1930), Morinaga and alli (1929), Smith (1934)
<i>E. Douglasii</i>	6	Winge (1925)
<i>E. caespitosa=tenuifolia</i> )	6	Winge (1929), Smith (1934)
<i>E. mollis</i>	8	Lawrence (1930)
<i>E. pulchella</i>	6	Sugiura

J. Dickson believed that *Eschscholtzia* was the most highly developed type of Papaveroideae (the writer's Cheridonioideae and Papaveroideae, cf. p. 574 phylogenic scheme) from his studies on the gynaecium of the Papaveroideae, but we can not agree with this from the karyological point of view.

### Chelidonieae

*Hylomecon japonica*. (Fig. 6) This plant is a unique species which is only grown in temperate East Asia. The pollen mother cells are about  $8.5 \mu$  in diam.. Six spherical meiotic chromosomes are found.

*Dicranostigma Franchetianum*. (Fig. 7) It is grown in Sze-Chuan and Yünnan and has 6 meiotic chromosomes like *Chelidonium*. The size of the chromosomes is equal to that of *Chelidonium majus*. M. E. Smith (1934), however, counted 16 somatic chromosomes, which seem to be doubtful.

*Chelidonium majus*. *Chelidonium* has only one species and some varieties. Since this has already been discussed in a previous paper, only certain interesting karyological figures are described here.

In the first metaphase it is often observed that there is a circular arrangement of chromosomes consisting of 6 at one pole and a 5-1 arrangement at the other. This suggests that the characters of both daughter nuclei are different. *C. majus* var. *laciniata* (Figs. 8a, 8b) which was studied anew, has also 6 chromosomes, which are as large as those of *C. majus*.

It is observed that although chromosomes are filar at the second anaphase, they are spherical at the first meta- and anaphase. Similar figures are also found in *C. majus*.

Probably this phenomenon may be due to the smaller viscosity of chromosomes at the second anaphase. Von Boenicke (1911) counted 8 meiotic chromosomes in *C. majus* and *C. laciniatum*, but to the writer it seems to be a miscount. Winge (1917) and Marchal (1920) also counted 6.

*Macleaya*

In this genus there are only two following species which are grown in Central Asia and temperate East Asia.

Eu-Macleaya .....	<i>M. cordata</i>	n = 10 (Fig. 9)
Pseudo-Bocconia .....	<i>M. microcarpa</i>	n = 10 (Fig. 10)

*Bocconia*

In this genus there are 9 species which are grown in Central and South America. I have studied one species, *B. frutescens* (Fig. 11).

Apart from systematic knowledge these three plants are very interesting karyologically. These 3 plants have the same number of meiotic chromosomes of almost the same shape and size of pollen mother cells in spite of the fact that they are grown in different localities of the world. Not only is their meiotic chromosome number 10 quite isolated from those of the neighbouring genera *Chelidonium*, *Hylomecon* etc. which have 6, but also their pollen mother cells are smaller than those of other genera in *Chelidoniae*. The chromosome number 10 has hitherto not been found in other genera in the *Papaveraceae*. Thus it is considered from the karyological point of view that *Macleaya* and *Bocconia* both branched out from the *Chelidonium*. It is possible that *Macleaya* and *Bocconia* were produced by the crossing of *Dicentra* or *Corydalis* and *Chelidonium* in early times, as the following expression shows:

$3 + 3 + 4 = 10$  where 3 represents *Chelidonioideae* and 4 *Corydaloideae* (see the writer's phylogenetic scheme p. 574).

*Papavereae**Glaucium*

This genus contains about 21 species which are grown around the Mediterranean. As there have been no karyological studies yet, the writer has studied the following three plants, the karyological accounts of which have been already published (Sugiura 1936).

*Glaucium corniculatum*, *G. flavum*, *G. flavum* var. *serpieri*. The meiotic chromosome number is 6 in these plants. As there are no different numbers of chromosomes to be found, the basic number is fixed at 3.

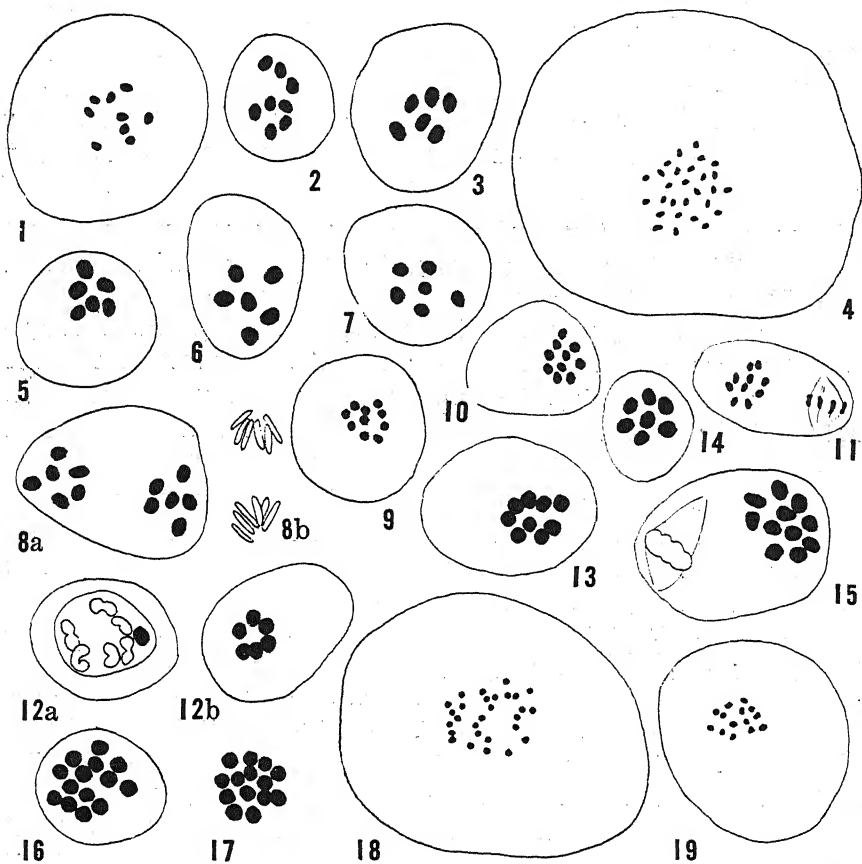
Generally plants belonging to *Papavereae* (the writer's *Papaveroideae* and *Glaucium*) have 7, 11 or their multiples (except *Roemeria* and *Glaucium*). It must be mentioned that, although *Glaucium* belongs to *Papavereae* it has only 6 meiotic chromosomes. In other words, *Glaucium* is not of *Corydaloid* descent, while other *Papavereae* are.

It is, therefore, not suitable to admit *Glaucium* as one of the genera of Papavereae (the writer's Papaveroideae and *Glaucium*) from the chromosomal point of view (cf. p. 574, phylogenic scheme).

J. Dickson, from his study of the gynaecium in Papaveroideae (the writer's Chelidonioideae and Papaveroideae), put *Glaucium*, *Sanguinaria*, *Chelidonium*, *Macleaya*, *Eschscholtzia* in his "*Glaucium*-group" and *Argemone*, *Meconopsis*, *Papaver* in his "*Papaver*-group". His idea coincides exactly with mine as stated above.

### *Roemeria*

This has 6 species. Lately Fedde (1936) has classified them into



Figs. 1-19. 1, *Pteridophyllum racemosum*. IM. 2, *Hypecoum procumbens*. IM. 3, *Platystemon californicus*. IM. 4, *Hunnemannia fumariaefolia*. IM. 5, *Eschscholtzia pulchella*. IA. 6, *Hylomecon japonica*. IA. 7, *Dicranostigma franchetianum*. IM. 8a, *Chelidonium laciniata*. IM. 8b, ditto. IIA. 9, *Macleaya cordata*. IA. 10, *Bocconia microcarpa*. IM. 11, *B. frutescens*. IIM. 12, *Roemeria violacea*. Diaphase. 12b, ditto. IM. 13, *R. speciosa*. IM. 14, *R. refracta*. IM. 15, *R. hybrida*. IIM. 16, *Meconopsis cambrica*. IA. 17, *M. nepalensis*. IM. 18, *Argemone grandiflora*. IM. 19, *A. barclayana*. IM.

Hybridoideae and Refractae, but I do not touch his classification at present.

Plants studied are as follows:

<i>Roemeria refracta</i> *	(Fig. 14)	n = 7	(4+3)
<i>R. speciosa</i>	(Fig. 13)	n = 9	(3+3+3)
<i>R. hybrida</i>	(Fig. 15)	n = 11	(4+4+3)
<i>R. violacea</i>	(Fig. 12b)	n = 6	(3+3)

It is very interesting to see that these plants each have different chromosome numbers, though their chromosome sizes are similar.

Karyologically they can be divided into two groups: one is a group of Corydaloidal descent, the other is a group of Cheridonioidal descent. This genus may be the ancestor of Papaveroideae (cf. p. 574, phylogenic scheme).

### *Meconopsis*

This genus, having about 45 species, distributed in Himalayan districts, France, England and Pacific North America, has not been studied karyologically.

<i>Meconopsis cambrica</i>	n = 14	<i>M. nepalensis</i>	n = 14
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Meiotic chromosomes are large in comparison with those of *Argemone*.

It is interesting to see that the meiotic chromosome arrangement (9-5 instead of normal 10-4) and the chromosome size are just the same in both species, although they grow in different districts, namely the former in M. and W. Europe and the latter in M. Asia.

### *Argemone*

This genus has 9 species which are grown in N. and S. America.

The writer has studied the following 3 plants, descriptions of two of them having already been published (1936).

<i>Argemone mexicana</i>	n = 14	<i>A. grandiflora</i>	n = 28 (Fig. 18)
<i>A. barclayana</i>	n = 14 (Fig. 19)		

Although the meiotic number 7 in this genus is still unknown, the basic number of chromosomes is probably 7. Meiotic chromosomes in *A. barclayana* are much smaller than those of *A. mexicana*. It is to be noted that the meiotic chromosomes in this genus are very much smaller than those of *Meconopsis* and *Papaver*, the adjoining genera.

This genus, belonging probably to the highest rank of Papaveroideae in America, has rather degenerated in comparison with the *Papaver* which is prosperous in Asia Minor as a centre.

\*) Plant names here enumerated are after those on the seed-packets from the Botanical Garden at Leyden.

### *Papaver*

This genus has about 90 species, which are grown mostly in the old world, but a few are native to W. and N. America. Unfortunately rather small number of karyological studies are to be found although most of the species are common as garden plants.

Karyological studies hitherto made of this genus may be shown in the following list:

Tahara (1915) on *P. rhoeas*, *somniferum* and *orientale*; Ljungdahl (1922) on *P. atlanticum*, *lateritium*, *persicum*, *tauricolum*, *hybridum*, *nudicaule*, *alpinum*, *rhoeas*, *pilosum*, *dubium*, *orientale*, *striatocarpum*, *radicatum* and *somniferum*; Yasui (1921-37) on *P. orientale*, *somniferum*, *bracteatum* and *lateritium*; Vilcins and Abele (1927) on *P. rhoeas*; Philip (1933) on *P. commutatum*; Lawrence (1930) on *P. rhoeas*; Kuzmina (1935) on *P. glaucum*, *setigerum*.

For years the writer has specially investigated the chromosome numbers in the genus *Papaver*.

In the following pages some observations on chromosome shapes and numbers will be given, together with remarks on the phylogeny of *Papaver*.

#### Sec. I. *Orthorhoeades* (about 43 species)

<i>Papaver rhoeas</i> v. <i>hookeri</i>	n = 7	<i>P. pinnatifidum</i>	n = 14
<i>P. commutatum</i>	n = 7	<i>P. dubium</i>	n = 21
<i>P. unbrosom</i>	n = 7	<i>P. pinnatifolium</i>	n = 14 (Fig. 21)

All these meiotic chromosomes are spherical and isomorphic. The pollen mother cells are rather larger than those of the other sections.

Philip (1933) also found 7 meiotic chromosomes in *P. commutatum*, and Ljungdahl (1922) counted 14 in *P. dubium*, whereas the writer found 21.

#### Sec. II. *Argemonorrhoeades* (about 6 species)

<i>Papaver argemone</i>	n = 21	<i>P. apulum</i>	n = 6 (5+L) (Fig. 22)
<i>P. hybridum</i>	n = 7 (Fig. 23)	<i>P. pavonium</i>	n = 6 (5+L)

*P. argemone* was discussed in a previous paper (Sugiura 1937b).

*P. hybridum*. The meiotic chromosome number is 7 which confirms Ljungdahl's count (1922).

*P. apulum*, *P. pavonium*. They have each 6 meiotic chromosomes, as reported in a previous paper (1937b). The number 6 is considered to have been derived from an original 7 by fusion of two chromo-

somes. This evidence is found in the diaphase, i.e. one pair of gemini out of 6 is two times long when compared with the others, and in the metaphase one of the chromosomes is larger than the others, especially in the case of *P. apulum*.

### Sec. III. *Carinatae*

The writer unfortunately has no materials belonging to this section, and can say nothing about it.

### Sec. IV. *Mecones* (about 6 species)

*P. glaucum*

n = 7

*P. setigerum* n = 11

*P. somniferum* v. *danebrog* n = 11 (Fig. 24)

Formerly the meiotic chromosome number was found to be 11 by Tahara and Yasui in *P. somniferum*, and 22 by Ljungdahl in *P. setigerum*.

In addition to the above, the writer reported 7 meiotic chromosomes in *P. glaucum* which attracted Kuzmina's attention. He, after examining the somatic chromosome number, decided that it was 14, and he had his doubt whether *glaucum* was the ancestor of *P. somniferum* and belonged to Sec. IV. *Mecones* or not. But the writer is inclined to accept Basileoskaya's opinion that *glaucum* and *gracile* are the progenitors of *somniferum*. This can be easily explained from the following: *glaucum* has 7 meiotic chromosomes consisting of 4 + 3; *somniferum* has 11 meiotic chromosomes consisting of 4 + 4 + 3 = 11, where 4 and 3 are the basic chromosome numbers in the Papaveraceae as will be explained afterwards. Quite independently, Yasui (1936, 37) also gave the chromosome constitution of *P. somniferum* (n=11) as  $4_{II}+3_I$  genetically in her studies of this species.

*P. setigerum* (Fig. 25). Formerly 22 meiotic chromosome numbers were counted by Ljungdahl and Kuzmina, but the writer now finds only 11. Now we note some quotations from Kuzmina's: "It is this wild growing species (*P. setigerum*) which is almost unanimously recognized by botanists as the progenitor of the cultivated poppy (*somniferum*)". "Crosses of *P. somniferum* and *P. setigerum* are, on the contrary, readily effected, and, according to the investigations of M. Vesselovskaya, give rise to perfectly normal fertile hybrids with viable seeds. The author, however, hesitates to recognize *P. setigerum* as a direct ancestor of *somniferum*, thinking it 'more correct to regard *P. somniferum* and *setigerum* as independent, though genetically near species having evidently arisen from one ancestor'."



Kuzmina also refused to admit that *P. setigerum* was a progenitor of *somniferum* in view of the fact that *setigerum* possesses 22 chromosomes in comparison with 11 in *somniferum*.

But now that meiotic number of chromosomes in *P. setigerum* has been proved by the writer to be 11, Kuzmina's conclusions need modifying.

It is also significant from the karyological point of view that *P. setigerum* is recognized by almost all botanists as the progenitor of *P. somniferum*.

In short, Kuzmina has denied that either *P. glaucum* or *setigerum* could be the ancestors of *somniferum*. But it seems to me that they are likely the ancestors of *somniferum*:

$$\begin{array}{ccccc} \textit{Papaver glaucum} & \longrightarrow & \textit{setigerum} & \longrightarrow & \textit{somniferum} \\ (3+4)=7 & & (3+4+4)=11 & & (3+4+4)=11 \end{array}$$

Here it seems to me that it is more necessary to explain 7 and 11 from the phylogenic as well as genetic point of view than from simple polyploidal, because a polyploidal study fails to explain how these numbers were produced.

#### Sec. V. *Miltantha* (about 15 species)

<i>Papaver armeniacum</i>	n = 7	(Fig. 26)	<i>P. floribundum</i>	n = 7	(Fig. 29)
<i>P. caucasicum</i>	n = 6 (5+L),	(Fig. 27b)	<i>P. persicum</i>	n = 7	(Fig. 30)
<i>P. desertorum</i>	n = 7	(Fig. 23)	<i>P. triniaefolium</i>	n = 14	(Fig. 31)

Among the above 6 species, *P. armeniacum* has not larger pollen mother cells and larger chromosomes than the rest.

*P. triniaefolium* has properly 14 meiotic chromosomes, but sometimes non-disjunction occurs during meiosis, i.e. 11 are found in one pole and the remainder in the other. Thus, the writer formerly mistakenly noticed that it had 11 meiotic chromosomes, but this non-disjunction of chromosomes has led to the conclusion that the meiotic number 14 consists of  $(4+3) \times 2$  and that 4+4+3 and 3 are separated.

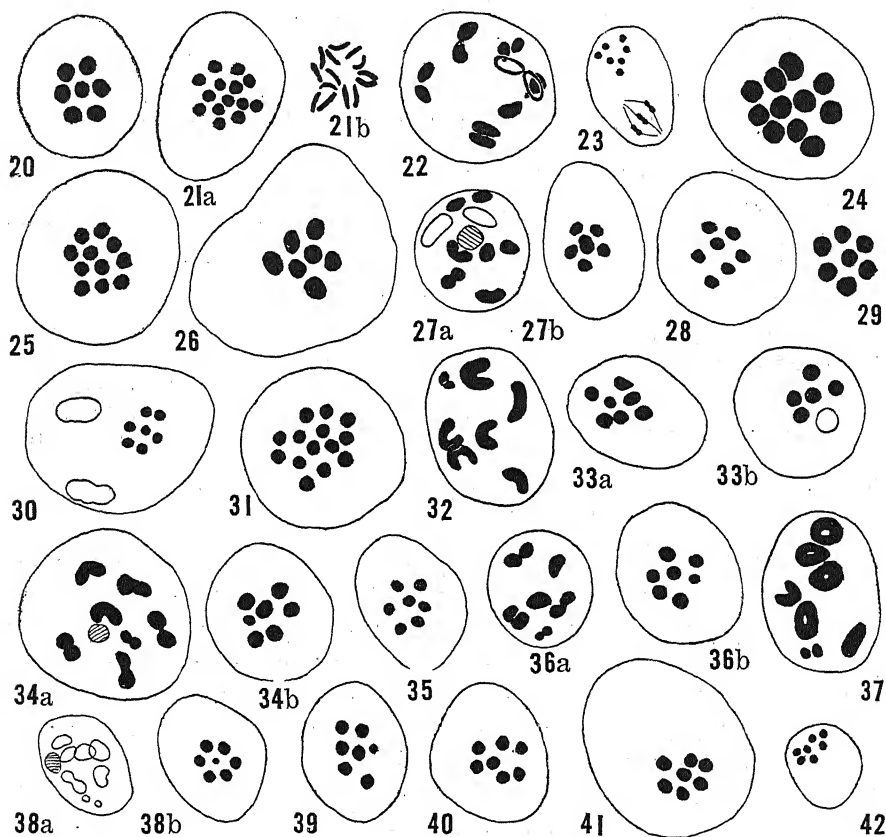
*Papaver caucasicum* has 6 meiotic chromosomes, one of which is twice the size of the others. The writer thinks that this phenomenon arises by fusing together of two chromosomes into one large one. This is clearly seen at diaphase, metaphase and anaphase. The similar case was also found in *P. apulum* and *pavonium*, though the latter show not so conspicuously the size difference in the metaphase and anaphase.

The large chromosome L, however, is not found in a fixed position. Sometimes it is in the center of a chromosome ring con-

sisting of 5, but sometimes as one member of the chromosome ring.

**Sec. VI. Pilosa (12 or more species)**

<i>Papaver atlanticum</i>	$n = 7$ (6+S) (Figs. 32, 33a, b)
<i>P. heldreichii</i>	$n = 7$ (6+S) (Figs. 34a, b)
<i>P. lateritium</i>	$n = 7$ (Fig. 35)
<i>P. oreophilum</i>	$n = 7$ (6+S) (Figs. 36a, b)
<i>P. pilosum</i>	$n = 7$ (6+S) (Fig. 37)
<i>P. schinzianum</i>	$n = 7$ (6+S) (Figs. 38a, b)
<i>P. strictum</i>	$n = 7$ (6+S) (Fig. 39)



Figs. 20-42. 20, *Papaver hookeri*. IM. 21a, *P. pinnatifolia*. IM. 21b, ditto. IIM. 22, *P. apulum*. Diaphase. 23, *P. hybridum*. IIM. 24, *P. somniferum* v. *danebrog.* IM. 25, *P. setigerum*. IIM. 26, *P. armeniacum*. IM. 27a, *P. caucasicum*. Diaphase. 27b, ditto. IM. 28, *P. desertorum*. IA. 29, *P. floribundum*. IA. 30, *P. persicum*. IIM. 31, *P. triniaefolium*. IA. 32, *Papaver atlanticum*. Diaphase. 33a, ditto. IA. 33b, ditto. IM. 34a, *P. heldreichii*. Diaphase. 34b, ditto. IM. 35, *P. lateritium*. IA. 36a, *P. oreophilum*. IA. 36b, ditto. IA. 37, *P. pilosum*. Diaphase. 38a, *P. schinzianum*. Diaphase. 38b, ditto. IA. 39, *P. strictum*. IM. 40, *P. burseri*. IM. 41, *P. nudicaule* v. *radicatum*. IM. 42, *Fumaria officinalis*. IA.

It is interesting to note that there are two kinds of chromosomes, normal and small (S), in the above species except *lateritium*.

Two kinds of gemini are also found at diaphase as shown in Figs. 32, 34a, 36a, and 38a. Among these the meiotic chromosome numbers of *atlanticum* and *pilosum* were also found by Ljungdahl to be 7 each. Consulting her studies and figures, it seems to me that she did not distinguish the two kinds of chromosomes found by myself.

In *P. atlanticum* there are two kinds of chromosome types one of which is 7 (6+S) (Fig. 33a) and the other 6 (5+L) (Fig. 33b), the latter number having been already reported (Sugiura 1936a, b). In those cases I wrote *P. rupifragum* for *P. atlanticum*, but it should be *P. rupifragum* v. *atlanticum*=*P. atlanticum*.

#### Sec. VII. Scapiflora (4 or more species)

*Papaver alpinum* n = 7

*P. nudicaule* v. *radicatum* n = 7 (Fig. 41)

*P. subsp. burseri* n = 7 (Fig. 40)

Formerly *P. alpinum* was investigated by Ljungdahl. She found 7 meiotic chromosomes which are confirmed by the writer.

*P. radicatum*, a variety of *nudicaule*, was also studied by her, 35 meiotic chromosomes being counted. The writer's count in the same plant is, however, 7. So we now know that there are two chromosome numbers in this plant, namely 7 and 35.

### Basic Numbers in Papaver

Up to this time two basic numbers have been found in the genus *Papaver*, but it has not been explained yet why there are two basic numbers in the *Papaver*. Darlington treated these numbers as uneven multiples. The writer, having studied Papaveraceae for some years, has concluded that *Papaver*, *Argemone*, *Meconopsis* and *Roemeria* are derived from both Corydaleae (the writer's Corydaloideae) and Chelidoniaceae (the writer's Chelidonioideae), the former having the basic number 4, and the latter 3. Thus the meiotic chromosome number 7 which is generally found in those genera of Papavereae (excl. *Glaucium*) (the writer's Papaveroideae) would be the sum of 4 and 3 (amphidiploidy). In the same way, we know that 11, which is found in the section *Mecones*, consists of 3+3+4. The same constitution of chromosomes was also explained from the genetical point of view by Yasui (1936 a, b, 37) as stated above.

From the chromosomal investigation of *Papaver* in eight sections the following can be said.

1. There are almost no significant differences in the sizes of

the pollen mother cells and of the meiotic chromosomes throughout 8 sections with some exceptions.

2. The chromosomes of different shapes are chiefly found in a certain limited section *Pilosa*.

3. The meiotic chromosome number 11, being found only in the sec. *Mecones*, is of special interest.

4. In no study of *Papaver*, including the present one, have meiotic chromosome numbers above 35 been found.

### Fumarioideae

#### Fumariceae

*Fumaria* (about 50 species)

*Fumaria officinalis* (Fig. 42). While Negodi (1935, 36a) and Wulff (1937) counted 14 meiotic chromosomes in this species, the writer has found only 7. Thus the basic number in *Fumaria* may now be put at 7. It is very interesting that, while other genera in Fumarioideae (the writer's Corydaloideae) all have 4 for their basic number, *Fumaria*, on the contrary, has 7. We may perhaps assume that the ancestor of *Fumaria* (*Corydalis*) has been crossed with that of Papaveroideae (the writer's Chelidonioideae) (*Glaucium*). According to the Wiesner's studies, "Fumarsäure" is found in *Fumaria*, *Glaucium* and *Corydalis*, and "Meconsäure" in the milky juice of *Papaver somniferum* and *P. rhoeas*. Hence we now know that *Glaucium* is directly connected with *Fumaria* and *Corydalis*. It can be said therefore that *Fumaria* must be derived from *Corydalis* and *Glaucium* in consequence of Wiesner's studies and my own. Thus it is believed that *Fumaria* is the youngest genus in Corydalo-Fumarioideae.

According to Hutchinson (1921) Fumarioideae (the writer's Fumarioideae and Corydaloideae) is an entirely independent family as there is no intimate relation between Fumarioideae (the writer's Fumarioideae and Corydaloideae) and Papaveroideae (the writer's Papaveroideae and Chelidonioideae). It is rather derived directly from the ancestors of Papaveraceae. Fumarioideae and Hypecoideae should be included (the writer's Corydaloideae, and Hypecoideae) and confronted to Papaveroideae (the writer's Papaveroideae and Chelidonioideae). Fedde (1936) is also of the same opinion. The idea will be also well supported from the chromosomal point of view if Papaverae (excl. *Glaucium*) (the writer's Papaveroideae) is excluded from Papaveroideae (the writer's Papaveroideae and Chelidonioideae) (cf. p. 574, phylogenic scheme). It may be noted that Honda and Sakisaka (1939) also treated these two subfamilies as two independent families.

## Corydaleae

*Corydalis* (nearly 300 species)

Chromosome numbers already found in the Corydaleae are as follows:

Plant names	n	2n	Authors	
<i>Corydalis cava</i>	8		Lawrence	1930
"	8		Tischler	1928, 29a
<i>C. lutea</i>		28	Kellett	1934
<i>C. pumila</i>	ca. 16*		Némec	1910a
<i>Dicentra pusilla</i>	8		Matuura and Sutô	1935
		16	Sakai	1934
<i>D. spectabilis</i>	8		Matuura	1935
<i>D. formosa</i>		24	Kellett	1934
<i>Adlumia cirrhosa</i>		32	Smith	1934

\* This number 16 varied from 12 to 20.

From the above list we now conclude that the basic number in Corydaleae (the writer's Corydaloideae) is 4.

Some of the data obtained in the present work, with regard to the number and size of chromosomes may be tabulated as follows:

Plants investigated	n	Chromosome length			
		IM( $\mu$ )	IA( $\mu$ )	IIM( $\mu$ )	IIA( $\mu$ )
<i>Pteridophyllum racemosum</i>	9	1.0×0.7			
<i>Hypecoum procumbens</i>	8	1.0×0.85			
<i>Hunnemannia fumariaefolia</i>	28	0.7×0.4			
<i>Eschscholtzia pulchella</i>	6		1.4×1.0		
<i>Hylomecon japonica</i>	6		1.5×1.25		
<i>Dicranostigma franchetianum</i>	6	1.0			
<i>Chelidonium laciniata</i>	6				2.5×0.36
<i>Macleaya cordata</i>	10		0.72		
<i>Bocconia microcarpa</i>	10	0.65×0.5			
<i>B. frutescens</i>	10			0.5×0.37	
<i>Roemeria refracta</i>	7	1.0			
<i>R. hybrida</i>	11			1.25	
<i>R. speciosa</i>	9		1.25		
<i>R. violacea</i>	6	1.125			
<i>Meconopsis cambrica</i>	14		1.25		
<i>M. nepalensis</i>	14	1.125			
<i>Argemone grandiflora</i>	28	0.5			
<i>A. barclayana</i>	14	0.5×0.25			
<i>Papaver hybridum</i>	7			0.6	
<i>P. hookeri</i>	7	1.15			
<i>P. somniferum</i> v. <i>danebrog</i>	11	1.75			
<i>P. setigerum</i>	11			1.25	
<i>P. armeniacum</i>	7	1.75			
<i>P. caucasicum</i>	6(5+L)		1.0; 1.25		
<i>P. desertorum</i>	7		1.125		
<i>P. floribundum</i>	7	1.3			
<i>P. persicum</i>	7			0.65	
<i>P. atlanticum</i>	7(6+S)		0.9; 0.37		
	6(5+L)	0.9; 1.5			
<i>P. heldreichii</i>	7(6+S)	1.4; 0.9			
<i>P. lateritium</i>	7			1.0	
<i>P. schinzianum</i>	7(6+S)		1.125; 0.45		
<i>P. strictum</i>	7(6+S)	1.1; 0.75			
<i>P. burseri</i>	7	1.1			
<i>P. radiculatum</i>	7	1.125			
<i>Fumaria officinalis</i>	7	0.5			

### Phylogenic Relation in Papaveraceae Based on Chromosome Numbers

Studies on the phylogeny of the Papaveraceae have been rather hypothetical and incomplete. As it has not been studied from karyological point of view yet, it may be of use to discuss it in the present paper. From the writer's studies it is clear that there are two basic numbers in the Papaveraceae, one 4, which is found in Hypecoideae and Fumarioideae (excl. Fumarieae) (the writer's Corydaloideae), and the other 3, which is found in Pteridophyllaceae and Papaveroideae except *Papaver*, *Argemone*, *Meconopsis* and *Roemeria* (the writer's Chelidonioideae). The basic number 7 is found in Papavereae, excluding *Glaucium* and some *Roemeria*. The secondary basic number 11 is often found in a section of *Papaver* and some *Roemeria*.

It is very interesting that the basic number in *Fumaria* is 7, which is otherwise not found in Fumarioideae (the writer's Fumarioideae and Corydaloideae). *Fumaria* is therefore probably derived from the ancestor in which Fumarioideae (*Corydalis*) and Papaveroideae (the writer's Chelidonioideae) (*Glaucium*) were united genetically.

While Léger (1894-5) has considered that *Fumaria* is the oldest genus in Fumarioideae and *Papaver* the most advanced in Papaveraceae, Fedde on the contrary thinks that Fumarioideae is the most advanced.

Hutchinson and Fedde have considered that Fumarioideae and Hypecoideae should be put together. To this proposal I agree, for it coincides with my karyological findings that these groups have the same basic chromosome numbers. *Hunnemannia*, belonging to Eschscholtzieae, has 7 for a basic number, while *Eschscholtzia* in the same group has 3. Therefore, probably, *Hunnemannia* was derived from an ancestor genetically connected with Fumarioideae (the writer's Corydaloideae) (*Corydalis* or *Dicentra*). Why we choose *Corydalis* or *Dicentra* here is that they are grown with *Eschscholtzia* and *Hunnemannia* in the same place.

So the chromosome constitution of *Hunnemannia* may be  $4 \times (3+4) = 28$  and *Hunnemannia* is considered younger than *Eschscholtzia*.

The same karyological considerations apply in the case of *Bocconia* and *Macleaya*. These genera, belonging to the Chelidonieae with *Chelidonium*, have 10 meiotic chromosomes, while *Chelidonium* has a basic number 3. 10 is therefore produced from  $3+3+4$ , 4 coming

from Fumarioideae (probably from *Corydalis* or *Dicentra* for the above reason).

*Glaucium* should be taken away from Papavereae for karyological reasons, since *Glaucium* has only 3 for its basic chromosome number, while other Papavereae have 7 or 11. J. Dickson puts *Sanguinaria*, *Glaucium*, *Chelidonium*, *Macleaya* and *Eschscholtzia* in his "*Glaucium* Group" and *Argemone*, *Papaver* and *Meconopsis* in a "*Papaver* Group" as a result of his study on the gynaeceum of *Papaver*. The writer agrees in this point with Dickson from the karyological point of view. *Roemeria* is a very interesting genus karyologically. My karyological studies on this genus show that there are various chromosome numbers as follows:

*Roemeria refracta*  $n = 7$  (4+3)

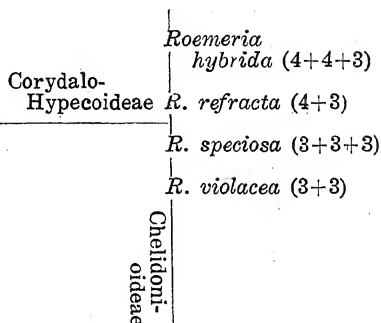
*R. violacea*  $n = 6$  (3+3)

*R. hybrida*  $n = 11$  (4+4+3)

*R. speciosa*  $n = 9$  (3+3+3)

From the above we know that *R. refracta* and *hybrida* have two kinds of basic numbers 4 and 3; on the contrary, *R. speciosa* and *R. violacea* have only one basic number, which is 3.

From these facts we conclude that the phylogenic relation of *Roemeria* is as follows:

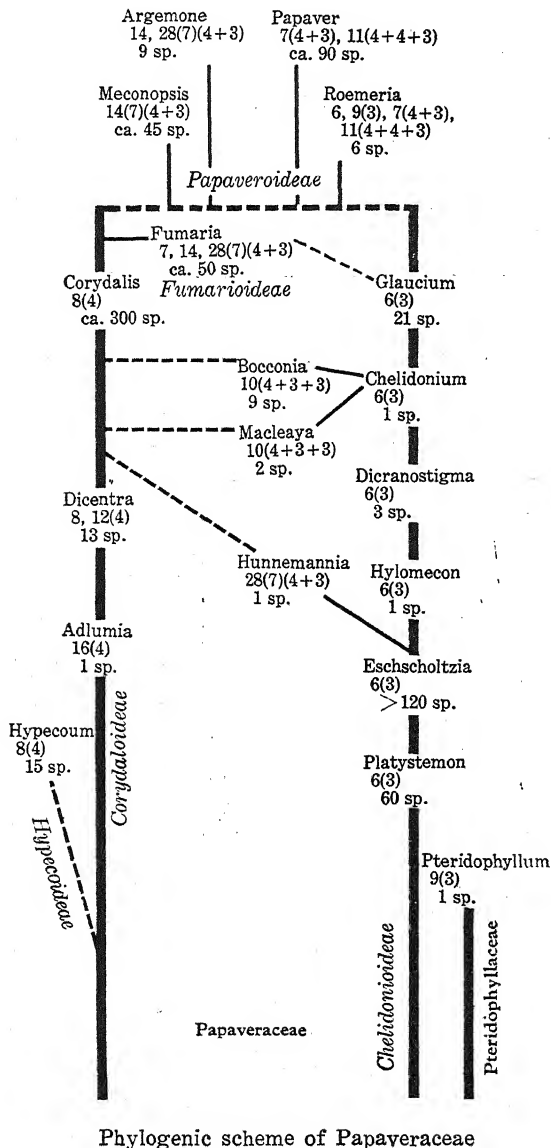


Karyological studies on *Papaver* show that the meiotic numbers of chromosomes are 7, 11 and their multiples. These numbers are probably derived from those of the ancestor in which Papaveroideae excluding *Papaver*, *Argemone*, *Meconopsis* and *Roemeria* (Chelidonioidae) and Fumarioideae (Corydaloideae, not the writer's Fumarioideae) united genetically ( $4 + 3 = 7$ ,  $4 + 4 + 3 = 11$ ).

But the union was so ancient that it seems difficult to trace it karyologically now. In some sections of *Papaver* there are 6 meiotic chromosomes, but they consist of 5 equal chromosomes and a big one, the latter being produced probably by fusing of two chromosomes as stated above. There are two parallel series in Papavereae, one of which is Corydalo-Hypecoideae with a basic number of 4 and the other Chelidonioidae with a basic number of 3.

And these two series unite to produce the writer's Papaveroideae. Thus it can be said that the writer's Papaveroideae is the most advanced subfamily in the Papaveraceae and the genus *Papaver*, having surpassingly more species than any other genera in Papaveroideae, is the most flourished genus in the Papaveroideae.

Léger also holds the opinion that *Papaver* is the most highly developed genus in the Papaveroideae. My karyological view quite coincides with his.



Phylogenetic scheme of Papaveraceae

It is probable that *Pteridophyllum*, having 9 meiotic chromosomes, a rare number in Papaveraceae, branched out from a very ancient ancestor of Papaveroideae (the writer's Chelidonioidae), as it is quite different from other papaveraceous plants not only in outer appearances but also in inner anatomical and karyological structures.

Thus it may be said that *Pteridophyllum* belongs to an independent family Pteridophyllaceae.

From the above descriptions and discussions we now present the new phylogenetic scheme as annexed. It is primarily based on Léger's (1894-5), but chromosomal considerations have led us largely to modify his scheme.

### Summary

- 1) Many meiotic chromosome numbers in the Papaveraceae have been counted.



2) *Pteridophyllum* is probably descended from a very remote ancestor of Chelidonioideae and should be put in the new family Pteridophyllaceae.

3) In the Papaveraceae there are two series, one of which, the Corydalo-Hypocoideae, having the basic chromosome number 4 and the other, Chelidonioideae the basic number 3. These two series are independent, but finally unite in Papaveroideae (e.g. *Roemeria*).

There are two kinds of *Roemeria*: one with the basic number 3 and the other with 3 and 4, thus showing that *Roemeria* is karyologically a heterogenous genus.

4) *Glaucium* which has been classified by Fedde with Papavereae, should be grouped with Chelidoneae (the writer's Chelidonioideae) as it is quite different karyologically from other genera in Papavereae.

5) We conclude from the present studies that the following genera have been produced by crossing between plants of Corydaloideae and Chelidonioideae, but their crossing occurred early in the history of development of the family, and we are at present not in a position to trace the exact chromosomal history.

<i>Hunneemannia</i>	7 = (4+3) .....	Corydaloideae	$\left( \begin{array}{c} \text{Corydalis,} \\ \text{Dicentra} \\ \text{or allied} \\ \text{species} \end{array} \right) \times$	<i>Eschscholtzia</i>
<i>Bocconia</i> <i>Macleaya</i>	10 = (4+3+3) .....	Corydaloideae	$\left( \begin{array}{c} \text{Corydalis,} \\ \text{Dicentra} \\ \text{or allied} \\ \text{species} \end{array} \right) \times$	<i>Chelidonium</i>
<i>Fumaria</i> (Fumarioideae)	7 = (4+3) .....	Corydalis or allied species $\times$ <i>Glaucium</i>		
Papaveroideae	7 = (4+3) 11 = (4+4+3) .....	Corydaloideae $\times$ Chelidonioideae		

\* In this table the writer's system and nomenclature are used.

6) *Papaver* can be said to be the most highly developed genus among the Papaveraceae, both for anatomical and karyological reasons.

7) Fedde is of the opinion that Fumarioideae (Fumarioideae and Corydaloideae of the present scheme) is the youngest in this family, but as stated before, *Fumaria*, having a different number of chromosomes from other genera in Fumarioideae (the writer's Corydaloideae), seems to be derived from a genetic connection of *Corydalis* and *Glaucium*, as *Fumaria* and *Glaucium* have fumaric acid in common.

The writer, therefore, believes that *Fumaria* is situated between *Roemeria* and *Corydalis*, having branched out from the latter. Other genera of Fumarioideae (the writer's Corydaloideae) also should

be placed before the Papavereae (the writer's Papaveroideae) because of their having the basic number 4.

8) *Argemone*, although it has fewer species as compared with *Papaver*, is the most advanced genus in America, while *Papaver* has become the most advanced genus in Europe and Asia Minor, having about 90 species.

In conclusion I desire to express my best thanks to Hon. Prof. Fujii and Prof. Nakai, the director of the Botanical Institute of the Tokyo Imp. University for much valuable advice and the great interest they have shown in my work, and also to express my sincere appreciation of the help and encouragement I have received from Prof. Sinotô and Dr. Yasui during the course of the present work.

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## Studies on the Influence of Ultraviolet Rays on the Cytoplasm, Tested by the Plasmolysis-Form Method

By

Noboru Takamine

*Received April 25, 1940*

### Introduction

According to the studies of various authors it is now believed that the exposure of cells to ultraviolet rays causes the increase of the viscosity of protoplasm and sometimes its coagulation.<sup>1)</sup> Ursprung and Blum (1917) have investigated the harmful effect of ultraviolet rays upon living plant cells by means of the plasmolysis and deplasmolysis method. So far as the writer's knowledge extends, no one has yet applied the plasmolysis-form method in the investigation of the influence of ultraviolet rays on protoplasm.

In the previous work the writer has tried to explain the genesis of different plasmolysis forms based on the assumption that it mainly depends upon the competition between the cohesion of the cytoplasm and its adhesion to the cell membrane. As this explanation could apply in various cases of the observations, in the present work the writer proposes to apply the plasmolysis-form method to test the nature of the cytoplasm influenced by ultraviolet rays.

A quartz mercury lamp of 100 volts and 3 amperes was employed, the distance between the lamp and the material being about 20 cm. In order to avoid any heat effect a water filter, whose wall was quartz plate, was placed between the lamp and material. The inner epidermis of scale leaf of *Allium cepa* was used as the material. After exposure of epidermis to the rays, as it is attached to the scale leaf, it was taken off from the latter, and then put in the plasmolyticum prepared on a slide glass. Chemicals, glass wares, water etc. were the same as described in the previous work<sup>2)</sup> and preparation also was made similarly.

When the monochromatic rays of 2535–2537Å and 3655Å were selectively used, they were dispersed by a quartz prism and each

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1) Hertel (1904), Schulze (1901), Vouk (1912), Cernovodeaun and Henri (1910), Ruppert (1924), Gibbs (1926), and Addoms (1927).

2) Takamine (1940).

ray of different wave length was converted by a lens on a quartz plate. The exact point of focus was detected by means of uranium glass plate. On the surface of epidermis, a piece of scale leaf was attached to the quartz plate on which the rays were focused.<sup>1)</sup>

In every experiment the exposure continued 45 minutes. The control was kept in a dark place otherwise under the same condition.

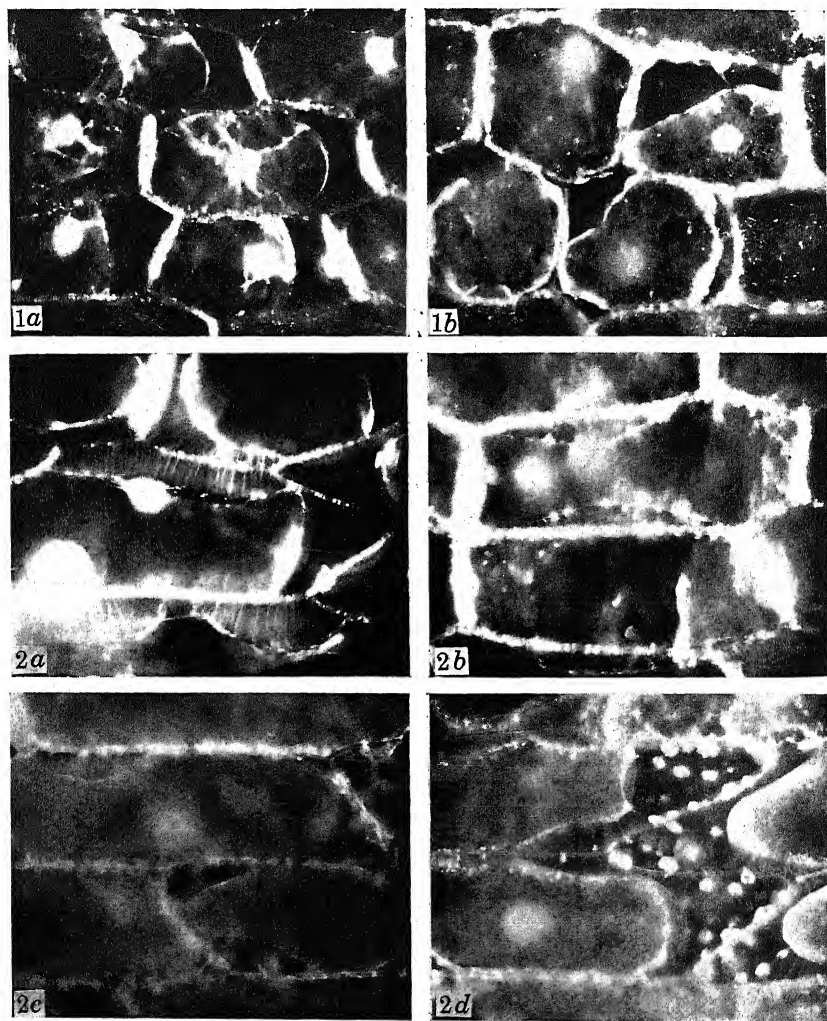


Fig. 1. Saccharose solution (0,8 mol). *a*, control; *b*, exposed. Fig. 2. KCl solution (0,5 mol). *a*, control; *b*, *c* and *d*, exposed.

1) The equipment is described in detail in an earlier paper (1935) by the present writer.

### Dark field observation

For the purpose of the precise observation of the plasmolytic feature it is sometimes advantageous to use a dark field illumination. In Table 1 the results of the observation of the plasmolyzed cells of the exposed material will be summarized together with those of the control. All the observations were made ten minutes after the immersion in the plasmolytica (Figs. 1-8).

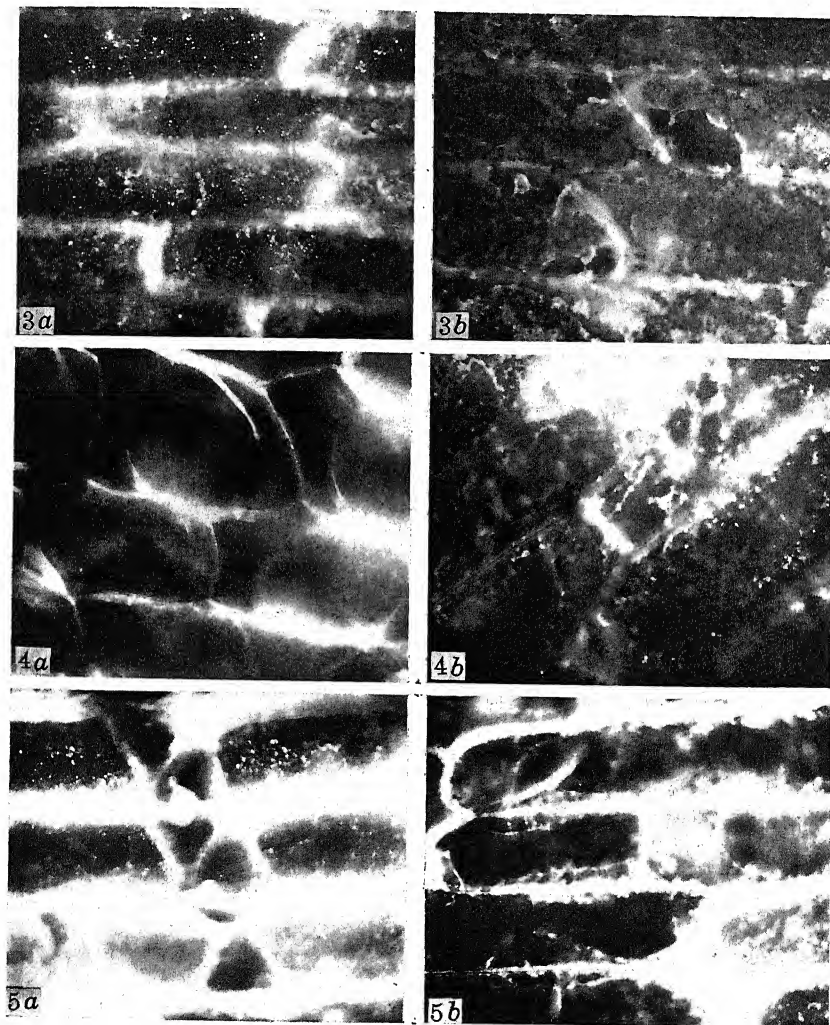


Fig. 3. KCl solution (0,5 mol). Cytoplasmic remains on the cell-membrane. *a*, control; *b*, exposed. Fig. 4. NaCl solution (0,5 mol). *a*, control; *b*, exposed. Fig. 5.  $\text{NH}_4\text{Cl}$  solution (0,5 mol). *a*, control; *b*, exposed.

In some of the experiments scale leaf was kept immersed in tap water during the exposure. In such cases no remarkable difference was found between the control and the exposed material,

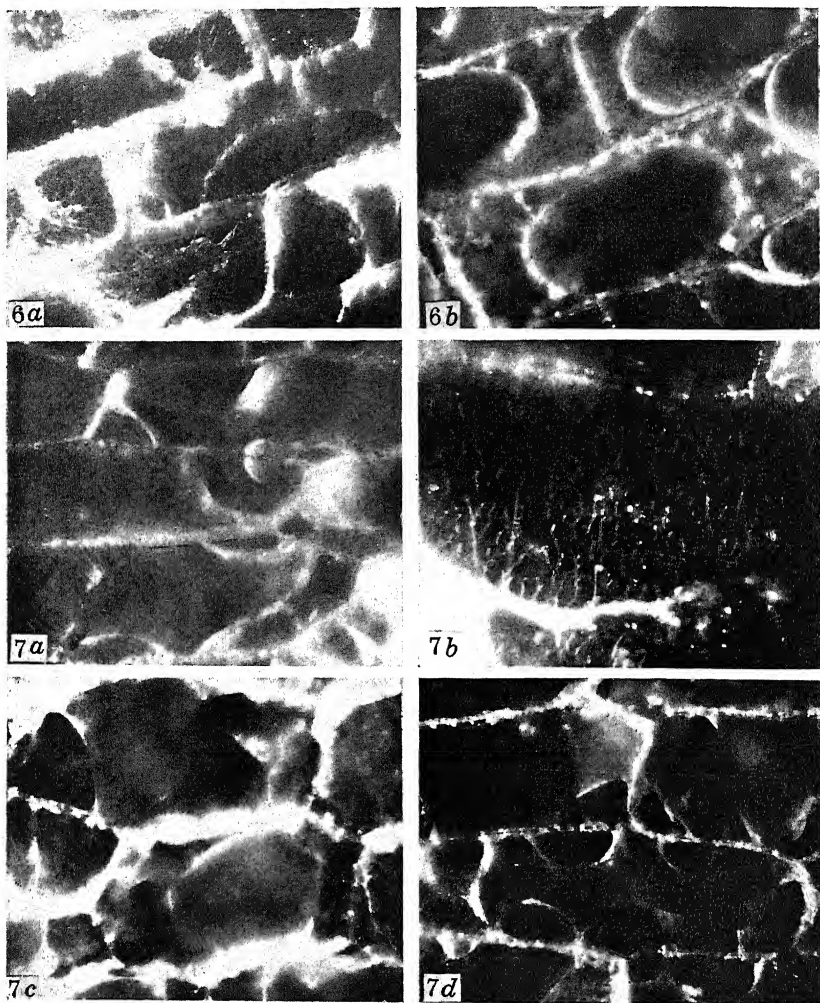


Fig. 6.  $MgCl_2$  solution (0,5 mol). *a*, control; *b*, exposed. Fig. 7.  $CaCl_2$  solution (0,5mol). *a*, control, *b*, control, wall patterns; *c*, exposed, disorganization of cytoplasm; *d*, exposed, weak disorganization.

when the cells were plasmolyzed in 0,5 m KCl solution (Fig. 9). In both cases fine Hecht's fibres and wall patterns appeared and frequently even streaming movement of the cytoplasm, which means probably the normal condition in the cell. Accordingly it should be remembered that the plasmolytic features treated here are limited to those which are found in the not immersed material.

Table 1

Plasmolyticum (mol)	Exposed	Control	Difference
Saccharose (0,8)	inclined to b	a <sup>1)</sup>	slight
KCl (0,5)	b	a	distinct
NaCl (0,5)	b	a	"
NH <sub>4</sub> Cl (0,5)	b	b	none
MgCl <sub>2</sub> (0,5)	b	a	distinct
CaCl <sub>2</sub> (0,5)	b, a	a	more or less distinct
AlCl <sub>3</sub> (0,5)	b, a <sup>2)</sup>	a	slight

a: Normal plasmolysis, showing Hecht's fibres and wall patterns.

b: Plasmolysis, more or less showing the tendency to tonoplast plasmolysis. Cytoplasmic particles remain on the cell membrane, scatter in the plasmolytic interspace or attach to Hecht's fibres. These particles are produced from the cytoplasm by violent plasmolysing force which acts to separate it from the membrane against the resistance of the strong adhesion.

The influence of the preliminary water immersion on the plasmolysis has been investigated by Höfler (1918, 1930) and others, and recently by Kamiya (1939) especially with the epidermis cell of *Allium cepa*. As such influence may not be negligible when the nature of the

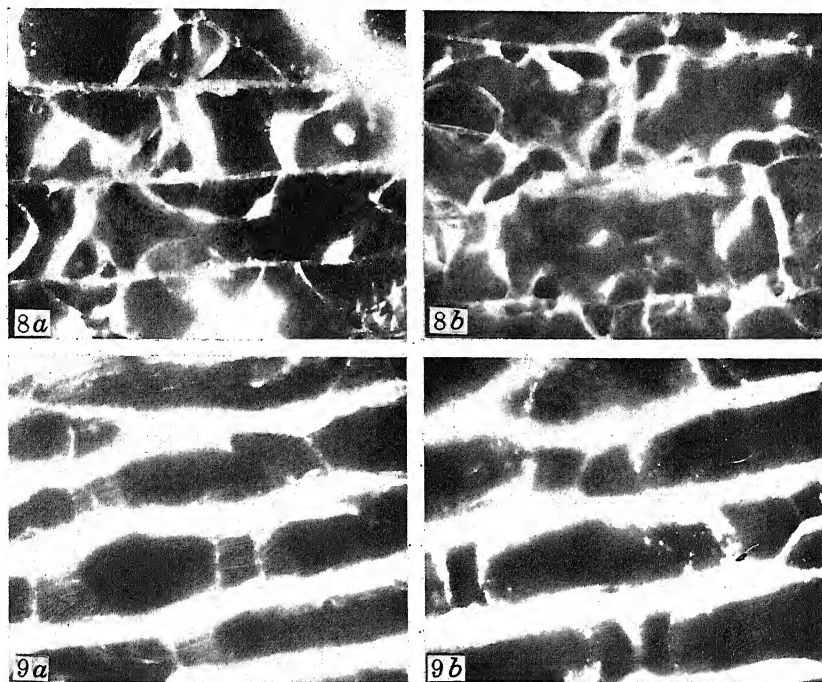


Fig. 8. AlCl<sub>3</sub> solution (0,5 mol). a, control; b, exposed, weak disorganization.  
Fig. 9. Water immersion during the exposure. Plasmolyzed in KCl solution (0,5 mol). a, control; b, exposed.

- 1) The cytoplasmic body separates smoothly from the cell membrane. Hecht's fibres and wall patterns are not visible.
- 2) Hecht's fibres are not distinct.



protoplasm is tested by means of the plasmolysis-form method, the writer is engaged now in experiments along this line.

When the material was exposed to the monochromatic rays of  $2535\text{--}2537\text{\AA}$  and  $3655\text{\AA}$ , some differences could be found between the control and the material exposed to the rays, but not between two rays of different wave lengths (Fig. 10). The plasmolytic features found in these exposed materials were likewise identical with those described in the case of the whole ultraviolet rays. Also in these experiments no special influence of the exposure could be found, if epidermis was kept immersed in water during the exposure.

### Ordinary microscopical observation

The results of the observations in the epidermis cells exposed to the ultraviolet rays, compared with those found in the control, are summarized in Table 2. The observations with each plasmolyticum was repeated ten times at least, and the result was always the same.

### Discussion

The main point of the assumption which the writer wants to adopt for the explanation of the genesis of several plasmolysis

forms is that they depend upon the competition between the cohesion of the cytoplasm and its adhesion to the cell membrane. The factors which facilitate the separation of the cytoplasm from the membrane easily cause a convex plasmolysis, while those which increase the adhesion have the tendency to keep the plasmolysis in

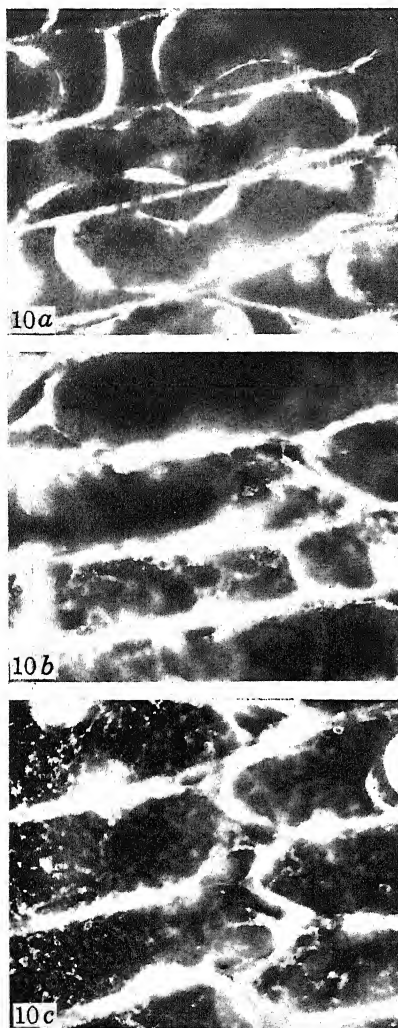


Fig. 10. Exposure to monochromatic rays. Plasmolyzed in KCl solution (0,5 mol). a, control; b,  $2535\text{--}2537\text{\AA}$ ; c,  $3655\text{\AA}$ .



Table 2

Plasmolyticum (mol)	Exposed	Control	Difference
Saccharose (0,8)	TC, TV	V	distinct
KCl (0,5)	TV, TC	C, TC	"
NaCl (0,5)	TV	C, V	"
Na <sub>2</sub> Cl (0,5)	TC, TV*	C, V, TV	"
MgCl <sub>2</sub> (0,5)	TV, TC*	C, V*	"
CaCl <sub>2</sub> (0,5)	TC, TV	C, V*	"
AlCl <sub>3</sub> (0,5)	TC, C*	C	more or less distinct

C: Plasmolysis begins in concave form and it lasts a pretty long time (Schema 1).

V: Plasmolysis begins in concave form, but it soon passes to convex form (Schema 2).

TC: Concave plasmolysis with the tendency to tonoplast plasmolysis (Schema 4).

TV: Concave plasmolysis with the tendency to tonoplast plasmolysis (Schema 5).

\*: Sometimes

concave form. If the cohesion or viscosity of the cytoplasm is anomalously lowered, for instance owing to high intrability for certain ions, or if the adhesion is relatively increased, the plasmolyzed cell shows more or less strong tendency to tonoplast plasmolysis, provided that the semipermeability of the tonoplast is kept without any change.

From the experimental results described above, it can be realized that the exposure to ultraviolet rays easily causes the formation of cytoplasmic granules and sometimes tonoplast plasmolysis. Therefore, it may not be impossible to conclude that this exposure acts to harden the surface of the cytoplasm and to intensify its adhesion to the membrane. This conclusion coincides with the opinions of many authors<sup>1)</sup> which have been reached from the facts that the exposure of cell to ultraviolet rays causes a cessation of the streaming movement of the cytoplasm, an increase of its viscosity and sometimes its coagulation. Based on this consideration the features of plasmolysis in the exposed material, compared with those in the control, may be explained without difficulty.

In the exposed cell it is easily noticeable that the plasmolyzed cytoplasm appears sparkling relatively in high degree in the dark field illumination, which may indicate its tendency to dehydration. Such optical feature is generally visible in any salt solution as well as in a saccharose solution; that is a phenomenon independent of the nature of the plasmolyticum.

In the cell plasmolyzed in a 0,5 mol KCl solution the cohesion of the cytoplasm is decreased owing to the easy penetrability of K<sup>+</sup> in the cytoplasm, and this is apt to cause a tonoplast plasmolysis even in the unexposed material. The adhesion increased by the exposure remains almost uninfluenced by K<sup>+</sup>. This unbalanced

1) loc. cit.

competition between two forces results in the peculiar plasmolysis form as mentioned above.

The observations made on the exposed cell plasmolyzed in  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$  and  $\text{MgCl}_2$  solutions can be explained in the same manner. Especially in a  $\text{NH}_4\text{Cl}$  solution the disorganization of the cytoplasm occurs remarkably and a tendency to tonoplast plasmolysis soon appears even in the control. This is due to the easy penetrability of  $\text{NH}_3$  or  $\text{NH}_4\text{OH}^{1)}$  which may be assumed more or less to exist on the surface of the cytoplasm as the result of hydrolysis of this salt in every moment.

In the case of  $\text{CaCl}_2$  the circumstance is a little different. The adhesion raised by the exposure is now once more strengthened by  $\text{Ca}^{++}$ , and this ion acts differently from  $\text{K}^+$  in the respect that the former is scarcely able to penetrate into the cytoplasm. Under this condition the cytoplasm is often subjected to a splitting force in the course of plasmolysis, but not so violently as in the case of  $\text{KCl}$ . The tendency to tonoplast plasmolysis is, therefore, not strong, if it occurs. In the unexposed material no remarkable change of the normal surface of cytoplasm can be expected when the cell is plasmolyzed in a  $\text{CaCl}_2$  solution, though the adhesion may naturally increase to some degree. In such cells the plasmolysis results rather in the normal concave form which sooner or later becomes the convex one.

The feature of the exposed cell plasmolyzed in an  $\text{AlCl}_3$  solution would be expected to appear similarly to that caused in a  $\text{CaCl}_2$  solution, because both equally belong to a group of salts whose cation is believed to dehydrate the cytoplasm and to be unable to penetrate into it. In fact, the disorganization of the cytoplasm is not remarkable, and it occurs weaker than in  $\text{CaCl}_2$  solution. In an  $\text{AlCl}_3$  solution the plasmolysis goes on very slowly and the separation of the cytoplasmic surface from the cell membrane occurs in many parts, besides each portion being very small. Such slow progress of the separation which is naturally attributed to the extreme intensification of the adhesion by  $\text{Al}^{+++}$ , that is, a large resistance against the plasmolytic contraction, and the dehydration of the inner part of the cytoplasm too, serves to save the cell from the violent disorganisation of its cytoplasm.<sup>2)</sup>

According to Heilbrunn and Daugherty (1933) ultraviolet radiation causes in amoeba cell a decrease in the plasmic viscosity

1) Otherwise this may be considered to be an ion pair  $\text{NH}_4^+$  and  $\text{OH}^-$ .

2) In the case of  $\text{Ca}^{++}$  the increase of the adhesion is not great enough and the adhesion and cohesion are not balanced enough to prevent the disturbance of the cytoplasm in the plasmolysis.

of the cortex or plasmagel and an increase in the viscosity of the interior protoplasm or plasmasol. In the present work, however, it is hardly possible to find any fact which indicates the liquefaction of the cytoplasm cortex, distinguished from the inner part.

Hecht's fibres appear in most cases hand in hand with wall patterns. Therefore, it is supposed that an intimate connection exists between the two features. Hecht (1912) considered that hyalo- and granular plasm take part in the appearance of these figures in epidermis cell of *Allium cepa*. In the same material Hansteen-Cranner (1922) and Weis (1925) followed the formation of the fibres, connected with the action of various cations. According to the former author Hecht's fibres are easily produced when Ca-salt is used as plasmolyticum, and Weis ascertained that the cytoplasmic precipitation sometimes occurs in the plasmolytic interspace in the following series:  $\text{NH}_4^+ < \text{K}^+ < \text{Mg}^{++} < \text{Ca}^{++}$ . Weber (1924, 1929) assumed that the viscosity of the cytoplasm is relatively low, when a convex plasmolysis occurs without Hecht's fibres, while it is high, when a concave plasmolysis or plasmoschisis occurs together with a plenty of plasmic fibres. With respect to Hecht's fibres and "plasmic tongue" Küster (1927) has remarked that, when he used concentrated solution such as a normal  $\text{KNO}_3$  solution as plasmolyticum, they appear very frequently.

According to the present writer's results Hecht's fibres are not remarkable in non-electrolyte plasmolyticum like cane sugar solution, but they appear in very fine form in various kinds of salt solution. The more healthy the material is, the more noticeably appear the fibres. The cytoplasmic surface stiffened by the ultraviolet ray exposure is inclined to refuse the formation of fine Hecht's fibres. If they are sometimes formed, a lot of cytoplasmic droplets are attached to them.

From the facts mentioned above the view that Hecht's fibres appear when the cytoplasmic surface is hardened, seems not always probable. They can not be formed by a violent separation of the cytoplasm from the cell membrane, but occur rather as a result of a gentle dismemberment of the thin surface film of the cytoplasm with which also the formation of wall patterns seems to be connected.

### Summary

1. In the cell of *Allium cepa* exposed to ultraviolet rays appear such features as cytoplasmic particles and tonoplast plasmolysis both of which mean the disorganization of the cytoplasm, when it is plasmolyzed. All these features which are caused in various

kinds of plasmolyticum, indicate the increase of the adhesion between the cytoplasm and the cell membrane.

2. Various features of plasmolysis in the exposed cell can be explained by an assumption of the competition between the cohesion of the cytoplasm and its increased adhesion to the cell membrane.

3. The ions or molecules which are believed to penetrate easily into the cytoplasm, facilitate the destructive process, for instance tonoplast plasmolysis, in the plasmolysis of the exposed cell, when their salts are used as plasmolytica.

4. Al-ion which acts further to raise the adhesion in the exposed cell, restrains the sudden separation of the cytoplasm from the cell membrane in the course of plasmolysis. This action serves more or less advantageously to protect the cytoplasm from the disorganization.

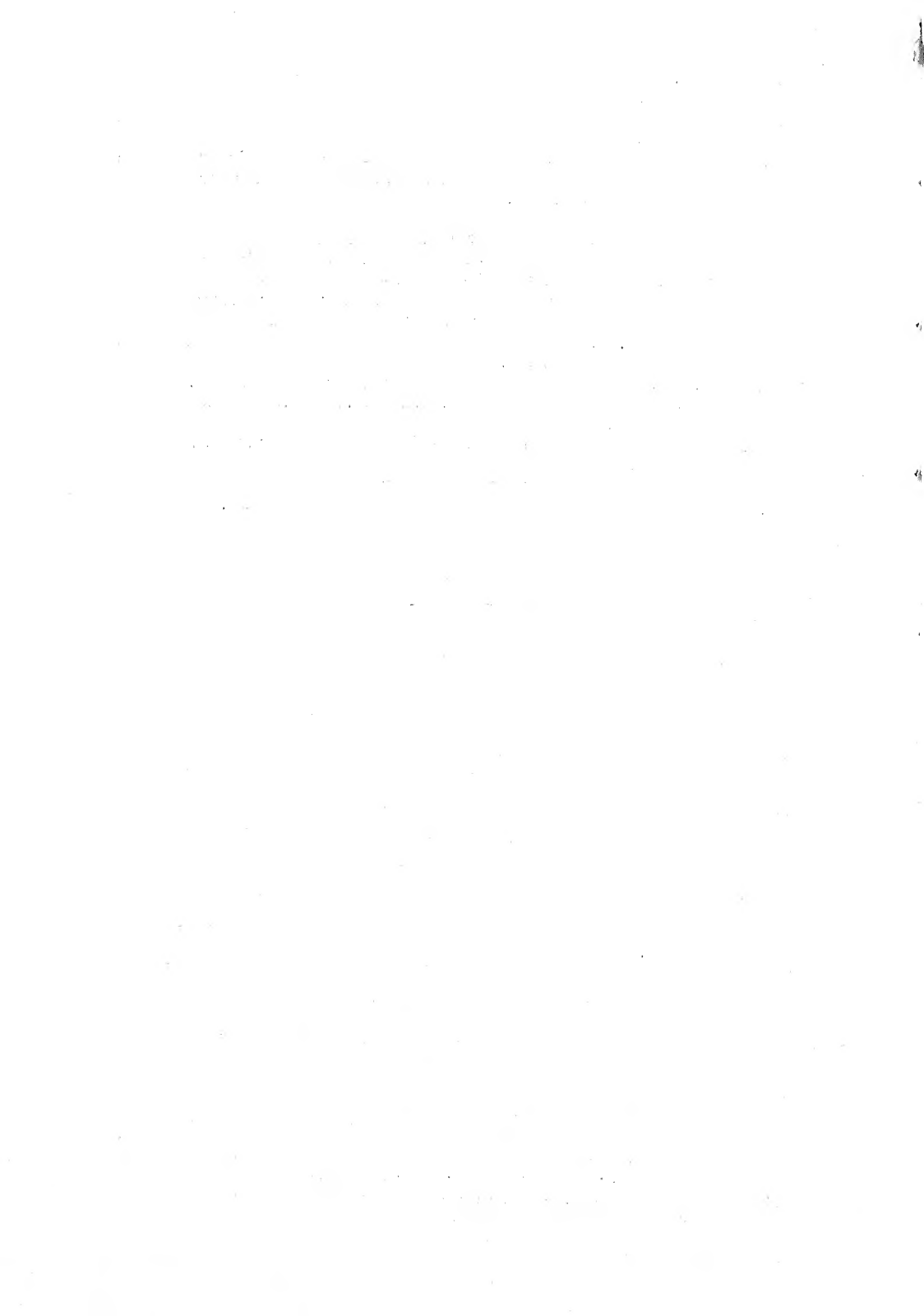
A part of the present study has been carried out at the Laboratory of Plant Physiology of Prof. T. Sakamura, Sapporo, to whom the writer wishes to acknowledge his sincere gratitude for granting him the facilities of the laboratory. The writer wishes to express his sincere thanks also to Dr. T. Hori, Professor of Physics, Sapporo, for his kind help in using the physical apparatus. The writer expresses his thanks equally to the Japan Society for the Advancement of Cytology, by the grant from which this work was supported.

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# CYTOLOGIA Vol. 10

## List of new publications

received by the Journal and the Editor from April 1939 to June 1940. Works not belonging to cytology and allied subjects are included only to a limited extent.

## Liste der Neuerscheinungen

die der Zeitschrift sowie dem Herausgeber von April 1939 bis Juni 1940 zugegangen sind. Arbeiten, die sich nicht auf die Zytologie und ihre Grenzgebiete beziehen, konnten nur in beschränkter Zahl aufgenommen werden.

## Liste des publications nouvelles

reçues par notre Revue et par son Directeur depuis avril 1939 jusqu'à juin 1940. Le travaux, qui ne concernent pas la cytologie et les sujets connexes, peuvent y être inclus, mais en nombre limité.

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